described by Dr. Chen in his Declaration at ¶¶ 6-12. These steps involve 1) culture of the biological sample in an isolation medium such as MacConkey agar, CLED agar, etc. for isolation and further biochemical testing; 2) growth of isolated colonies on a nonspecific medium; 3) biochemical testing of suspected colonies to definitively identify them; 4) antimicrobial susceptibility testing. This process usually takes 3-4 days and requires skilled workers to perform it.

The present invention is a marked improvement over the prior art because it reduces the test protocol to a single step that can be done in 24 hours with workers of a much lower skill level. The present invention provides a uropathgenic specific medium, which allows for the substantial growth of only the primary gram negative uropathogens. The prior art does not teach or suggest such a medium. The MacConkey agar referred to by the Examiner is simply an isolation medium for the isolation of gram negative bacteria for later biochemical identification. The present invention also provides an antimicrobial susceptibility interpretation medium. Thus is another claimed element not taught or suggested by any reference(s) cited by the Examiner.

Johnson discloses only a device for use in exposing a test sample to a variety of antibiotics and for determining the susceptibility of microorganisms to those antibiotics. The device has multiple growth wells in which concentrations of suitable agents are predeposited and dried and rehydrated with inoculum (Col. 2, lines 27-34). The figures of the Johnson reference provide illustrations of the device. **Johnson does not describe any growth medium whatsoever**. Thus, any medium to be used in the device must be those available in the prior art or otherwise provided by the user.

Libman teaches a device for collecting body fluids, particularly the mid-stream collection of urine (Col. 1, lines 66-68), and for inoculating a bacterial medium with a

body fluid soon after the fluid leaves the body (Col. 2, lines 4-7). **Libman does not describe any growth media whatsoever.** Libman merely refers to growth media of the prior art, particularly at Col. 3, line 64 – Col. 4, line 15. Libman teaches the use of CLED agar, MacConkey agar, or EMB agar. Libman states that CLED agar is "ideal in enumerating and presumptively identifying urinary flora." But Libman clearly states at Col. 4, lines 3-4 that CLED agar "supports growth of urinary pathogens **and contaminants.**" Therefore, Libman teaches that the "ideal" medium (i.e., the best available) for enumerating and presumptively identifying urinary flora allows the growth of contaminants. Libman also teaches what is already known by microbiologists -- that these media are isolation media for isolation of colonies for further biochemical testing. These statements show that CLED medium and MacConkey agar allow for the substantial growth of organisms other than the primary gram negative uropathogens. Therefore, none of the media referred to by Libman can **definitively** identify uropathogens. Instead, these media function according to the prior art process generally comprising four steps, as explained by Dr. Chen in his Declaration at ¶ 6-12 and above. Therefore Libman does not teach a uropathogenic specific medium as that term is used in the specification (See, e.g., p. 12). Therefore the present invention is not obvious over Libman or the inappropriate combination of references asserted by the Examiner.

Media that allow for the growth of contaminating organisms cannot function in the present invention because they will result in many false positives, and therefore unnecessary treatment of patients who do not have a urinary tract infection caused by one of the primary gram negative uropathogens. This problem is especially acute when testing specimens collected in a veterinary context from an unwilling animal patient. Such samples frequently are contaminated during the collection process. For example,

samples collected in the veterinary context often must be pipetted off of the floor. In addition, obtaining samples from a urine stream is often difficult and samples often become contaminated by contact with the patient's fur. For these reasons, the high specificity of the medium is important, and isolation media merely for presumptive identification are not appropriate in the present invention.

The Examiner is incorrect in stating that "no teaching or recitation is found in the originally filed specification or claims for specific exclusion of gram negative bacteria which are not primary gram negative uropathogens." (Office Action mailed 9/26/00, p. 6). See, e.g., p. 12 which refers to a "uropathogenic specific medium" as one that allows only the growth of the primary urinary gram negative pathogens and allows for substantially less growth of any other bacteria of a biological matrix. The Applicants also note that they are entitled under the patent laws to claim the media based on what it does, i.e., to claim the invention functionally. *In re Swinehart*, 169 U.S.P.Q. 226 (CCPA, 1971). Therefore, the Examiner's statement at p. 7 that the claims do not recite "antibiotics in a selective medium" is irrelevant when the limitation is claimed functionally. *Id.* at 212.

In making this rejection the Examiner has inappropriately combined Johnson and Libman with the Manual of Clinical Microbiology. The Manual of Clinical Microbiology actually teaches away from this invention and supports the Applicant's points stated above. Referring to urinary tract infections at p. 264, left column, the Manual teaches that "Because of the severe nature of many of these infections, prompt isolation, identification, and susceptibility testing of Enterobacteriaceae are essential." In the right hand column the Manual continues "Urine specimens should be collected by methods that reduce the possibility of contamination of the specimen. Such methods

include the clean-catch, mid-stream urine technique, catheterization under aseptic conditions, and in certain instances, suprapubic aspiration." In the present invention, the "isolation, identification, and susceptibility testing" are performed in one step. And the sterile collection methods are unnecessary because the present invention provides a uropathogenic specific medium that allows for the substantial growth of only the primary gram negative uropathogens. The present invention is designed to test urine samples that have been contaminated with non-target organisms from the environment, and that this contamination does not change the result achieved. It is apparent from the cited passage that the media taught by the Manual are not sufficient to test contaminated urine samples.

Furthermore, at p. 264, bottom right column, the Manual teaches that MacConkey agar allows for a "**preliminary** grouping of enteric and other gram-negative bacteria." These passages indicate that MacConkey agar allows for the substantial growth of contaminating organisms.

Additional evidence is found at page 265, bottom of the left column, which states: "Members of the family Enterobacteriaceae are identified primarily by testing for biochemical reactions." This is the prior art method of using biochemical testing to identify bacteria described by Dr. Chen at ¶ 10 of his Declaration. If MacConkey agar allowed for the substantial growth of only the primary gram negative uropathogens (i.e., was a uropathogenic specific medium), this testing would not be necessary because an organism growing on the medium would be automatically identified.

The Examiner asserts that one of ordinary skill in the art would expect success in using MacConkey agar (Office Action mailed 9/25/00, pp. 5-6). As explained by Dr. Chen in ¶ 13 of his Declaration, MacConkey agar provides a signal based on the fermentation of lactose and the consequent drop in pH, which causes a dye in the

medium to provide a color change. But some primary gram negative uropathogens do not ferment lactose (e.g., *Proteus*), and therefore MacConkey agar does not provide a signal when these organisms are present. Furthermore, the present claims have been amended to recite that the presence of urinary pathogens is indicated in the medium by "metabolism of a signal generating substrate and production of a detectable signal." (See p. 13, lines 9-31 of the specification). Since MacConkey agar does not produce a detectable signal when *Proteus* is present, MacConkey agar is not within the scope of these claims.

For the reasons explained, reconsideration and withdrawal of the rejection are respectfully requested.

**2. Claim 26 is rejected under 35 U.S.C. 103(a) as being unpatentable over Johnson in view of Libman and the Manual of Clinical Microbiology as applied to claim 20, and further in view of Brocco.**

This rejection is respectfully traversed. Combining Brocco (inappropriately) with the previous references cited with respect to claim 26 does not cure the deficiencies noted above.

Brocco discloses methods for assaying for microorganism growth or inhibition in the presence of antibiotic. Brocco does not disclose any selective media. In the method of Brocco, it is necessary to use a sterile device and sterile medium which contains a particular antibiotic, and to inoculate it with pure cultures of pre-grown bacteria (Brocco, p. 4, lines 3-11). This is done for the purpose of evaluating the susceptibility of those bacteria to particular antibiotics. **If the medium or device is not sterile, any contaminating bacteria will give a false result because, unlike the present invention, the medium of Brocco are not selective for any bacteria, and therefore many types**

**of bacteria will grow in the medium**: The result can only be correlated to the bacteria tested if the medium was inoculated with a pure culture of that bacteria. **In the present invention, these sterility concerns are unnecessary because the medium is selective for the primary gram negative urinary pathogens, i.e., only this class of bacteria will grow in the media.**

One can not simply add antibiotics to a medium and presume that one will arrive at a medium that appropriately tests the susceptibility of an entire group of organisms. This is due to antibiotic cross-reactivity described by Dr. Chen at ¶¶ 18-19 of his Declaration. Different combinations of antibiotics and medium ingredients frequently yield very different resistance patterns. A bacteria susceptible to an antibiotic in one medium frequently is not susceptible to the same antibiotic in another medium. And adding additional antibiotics to a medium may reverse a bacteria's susceptibility to an antibiotic it was previously susceptible to in the medium.

Furthermore, the cultures of Brocco are pure cultures. These are completely different from the presently claimed invention, which allows the user to take a sample of urine containing both target uropathogens and contaminating organisms, and test for the presence of uropathogens and **simultaneously** determine the susceptibility of a particular uropathogen to a given antimicrobial agent. The present invention also allows this to be done in only 24 hours as opposed to 2-3 days required by prior art methods.

For the above reasons, and those further explained in the Declaration of Chun-Ming Chen, the combination of references cited by the Examiner do not teach or suggest a method of detecting the presence of the primary gram negative uropathogens, and of simultaneously determining the susceptibility of those pathogens to antimicrobial agents.

Reconsideration and withdrawal of the rejections is respectfully requested, and that the claims be passed to allowance.

## *Conclusion*

All of the stated grounds of objection and rejection have been properly traversed, accommodated, or rendered moot. Applicants therefore respectfully request that this application be passed to allowance. If the Examiner believes that personal communication will expedite prosecution of this application, the Examiner is invited to telephone the undersigned at the number provided.

Prompt and favorable consideration of this Amendment is respectfully requested.

Respectfully submitted,

Date: March 26, 2001

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**Marked up version of claim**

20. (Amended) A method of detecting the presence or urinary pathogens in a biological sample and of simultaneously determining the susceptibility of the urinary pathogens to antimicrobial agents, said method comprising:

providing a multicompartment assay device comprising: at least one compartment comprising a medium capable of sustaining growth of total microbial organisms; at least one compartment comprising a uropathogenic specific medium; and, at least one compartment comprising an antimicrobial susceptibility interpretation medium;

placing a portion of the biological sample respectively in said at least one compartment comprising a medium capable of sustaining growth of total microbial organisms; said at least one compartment comprising a uropathogenic specific medium; and, said at least one compartment comprising an antimicrobial susceptibility interpretation medium comprising an antimicrobial agent;

whereby metabolism of a signal generating substrate and production of a detectable signal [growth of organisms] in said at least one compartment comprising a medium capable of sustaining growth of total microbial organisms indicates the presence of microbial organisms in the sample; metabolism of a signal generating substrate and production of a detectable signal [growth of organisms] in said at least one compartment comprising a uropathogenic specific medium indicates the presence of urinary pathogens in the sample; and metabolism of a signal generating substrate and production of a detectable signal [growth of organisms] in said at least one compartment comprising an antimicrobial susceptibility interpretation medium indicates that the organisms lack susceptibility to the antimicrobial agent comprised in said antimicrobial susceptibility interpretation medium; and

examining the compartments to determine the presence of urinary pathogens in said biological sample and the susceptibility of said urinary pathogens to said antimicrobial agents.

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE



In re application of:

**Chun-Ming Chen, Charles Carpenter, Haoyi Gu, and Ali Naqui**

#33 Plunkett 4/7/01

Art Unit: 1623

Appl. No. 08/942,369

Examiner: M. Moran

Filed: October 2, 1997

Atty. Docket: 03604-0010-US00

For: **METHOD AND APPARATUS FOR CONCURRENTLY DETECTING PATHOGENIC ORGANISMS AND ANTIMICROBIAL SUSCEPTIBILITY**

## Declaration of Chun-Ming Chen, Ph.D. under 37 C.F.R. § 1.132

Commissioner for Patents
Washington, D.C. 20231

Sir:

1. I, Chun-Ming Chen, hold a Ph.D. in Food Science from Rutgers University, New Brunswick, New Jersey. I also hold a Master's degree from the University of Florida, Gainesville, Florida in Food Science. I have worked at the Food and Environmental Division at IDEXX Laboratories as a microbiologist since 1993. I am the co-author of three patents in the area of microbiology, six peer-reviewed publications in the area of biochemistry and microbiology and another sixteen publicly-presented abstracts. A recent resume is attached to this Declaration.

2. I understand that the Examiner has cited references by Johnson, Libman, and the Manual of Clinical Microbiology[1] in this case. I am a co-inventor of the above

1 Manual of Clinical Microbiology, Fourth Edition, (ed.-in chief, Lennette, E.H.), American Society for Microbiology, Washington, D.C. (1985).

referenced patent application. I have reviewed the specification, the present claims, and each of the references cited and believe that a person of ordinary skill in the art would not find the presently claimed invention obvious over any of these references, nor any combination of them.

**The Present Invention**

3. This invention involves methods for detecting the presence or absence of urinary pathogens in a biological sample and for **simultaneously** determining the susceptibility of the pathogens to various antimicrobial agents (specification, p. 7, line 21 – p. 8, line 26). The present invention accomplishes these objectives by providing a **uropathogen specific medium**, i.e., a medium which allows substantially only the growth of the primary urinary gram negative pathogens and allows for substantially less growth of any other bacteria of a biological matrix AND by incorporating within that medium an antimicrobial agent to evaluate its effectiveness against gram negative pathogens (specification p. 12, line 11 et seq.; p. 19, Table 1). In this context, the term "**primary gram negative urinary pathogens**" refers to the group of bacteria which cause at least 85-90% of the human and veterinary urinary tract infections (specification, p. 10, line 19 et seq.).

4. The medium of the present invention is provided in a multicompartment assay device with at least three compartments containing, respectively, 1) a medium capable of sustaining the growth of total microbial organisms (i.e., a control); 2) a uropathogen specific medium (which allows for the growth only of the primary urinary gram negative pathogens); and 3) antimicrobial susceptibility interpretation medium (which comprises the uropathogen specific medium with an antimicrobial substance(s) to be evaluated for their effect on the urinary pathogens) (p. 7, line 21 – p. 8, line 26).

**The Obstacles Faced by the Person of Ordinary Skill**

5. The process presently used in the art of microbiology for identifying bacteria and evaluating their susceptibility to antimicrobial agents involves four steps.

6. **STEP 1**: The sample must be plated out on an isolation medium such as MacConkey agar that allows for the growth of a limited group of organisms. Exhibit 1 is an excerpt from the Difco Manual[2]. The manual states the following at page 288 under "Intended Use":

> MacConkey Media are selective and differential plating media mainly used for the detection and isolation of gram-negative organisms from clinical, dairy, food, water, pharmaceutical and industrial sources.

7. The purpose of this step is the isolation of the organisms for performing further biochemical identification tests Many contaminating organisms can be identified and screened out in this step based on the appearance of the colonies. Colonies growing on the medium that appear to be of interest are passed into Step 2. The MacConkey agar referred to by the Examiner (Office Action mailed 9/25/00, pp. 5-6) differentiates organisms based on their ability to ferment lactose. When lactose is fermented in the medium, it causes a consequent drop in the pH of the medium. Not all primary gram negative organisms have the ability to ferment lactose. For example, *Proteus spp.* does not have this ability and will not hydrolyze or convert the signal generating substrate to produce a signal when growing on MacConkey agar. MacConkey agar is inappropriate for use in the present invention because it will result in false positives and/or false negatives, for reasons further explained below.

2 Difco Manual (11th ed), Difco Chemical Corp.

8. **STEP 2**: The organisms selected by the MacConkey agar are then grown on a nonspecific medium. The purpose of this step is to **generate isolated colonies.** Once isolated colonies are grown, these colonies may be subjected to biochemical testing in Step 3 to determine their identity.

9. **STEP 3**: In this step, the identity of the pure colonies from Step 2 is determined using biochemical testing. This testing involves a battery of biochemical tests in order to ascertain the identity of the colonies that grew on the MacConkey agar.

10. Attached as Exhibit 2 is a copy of pages from the Manual of Clinical Microbiology, 7th ed.[3] The passage at page 453 indicates that a number of biochemical screening tests is necessary even for "tentative" identification of Enteroacteriaceae. Table 6 of the reference lists several of those tests. This is consistent with my knowledge in the field that MacConkey agar is an isolation medium for isolation of colonies for biochemical analysis. Growth and signal production on MacConkey agar indicates that the organisms are lactose fermenters. One cannot properly conclude that simply because a colony is growing on MacConkey agar that it is a primary gram negative urinary pathogen, and scientists do not use isolation media such as MacConkey agar for this purpose due to the unreliability of such conclusions. For example, MacConkey agar allows the growth of both lactose fermenters and non-lactose fermenters. But non-lactose fermenters will not produce a signal since the signal generation in MacConkey agar is dependent on the fermentation of lactose and the consequent drop in pH. *Proteus spp.* is an example of an organism that will grow on MacConkey agar but not produce a signal. *Proteus spp.* is a primary gram negative uropathogen that does not ferment lactose.

3 Manual of Clinical Microbiology, 7th edition, (ed. Murray et al.), ASM Press (Washington, DC, 1999)

11. **STEP 4**: Finally, the purified colonies that have been definitively identified as primary gram negative urinary pathogens based on biochemical testing are placed in a nonselective medium containing an antimicrobial agent. If the colony fails to grow in this medium, the organism may be susceptible to the antimicrobial agent in the living human or animal. The process of carrying out these steps takes 3-4 days and requires the use of skilled workers.

12. The present invention provides a medium that is substantially specific for the primary gram negative uropathogens. This medium is able to definitively identify the primary gram negative uropathogens because the medium allows for substantial growth of only these organisms. This is one aspect of the invention that, by itself, is not obvious to one of ordinary skill in the art because it is not taught or suggested by any prior art reference or combination of references.

13. I understand the Examiner has asserted that the presently claimed invention might encompass MacConkey agar. This is incorrect. As noted earlier, signal is generated in MacConkey agar based on fermentation of lactose and a consequent drop in pH, which causes signal production. MacConkey agar is not functional in the present invention because not all of the primary gram negative uropathogens have the ability to ferment lactose (e.g. *Proteus spp.*). Therefore, in an unacceptably high number of cases false negatives would occur because target organisms would be growing and no signal would be generated.

14. Development of a uropathogenic specific medium required inventive work, but this represents only one of the inventive aspects of the present invention. As noted above, to arrive at the present invention it was necessary to incorporate within the uropathogenic specific medium an antimicrobial agent. The purpose of this is to enable

the medical practitioner to evaluate the susceptibility of the particular strains of uropathogens involved in an infection to various antibiotics. Therefore, with the present invention, the user may simultaneously 1) **definitively** determine whether primary gram negative uropathogens are present in the sample; and 2) if present, identify the antimicrobial agents to which these uropathogens will be susceptible.

15. Therefore, **with the present invention, the four steps of the prior art methods (which require 3-4 days to perform), have been reduced to ONE step that is performed within 24 hours**. In the present invention, primary gram negative uropathogens are definitively identified and their susceptibility to at least three antimicrobial agents is evaluated SIMULTANEOUSLY. Thus, in a matter of only 24 hours the user can determine whether uropathogens are present, and if so, the antimicrobial agents to which they are susceptible. The prior art methods using the four steps described above requires 3-4 days of testing and skilled personnel. Therefore, the present invention provides this information 2-3 days faster than the prior art methods, and reduces the four labor-intensive steps described above to a single simple step, which can be performed by a worker with a far lower skill level than required by prior methods.

16. The present invention involves: 1) determining a particular combination of medium ingredients that will allow for the substantial growth of the primary gram negative uropathogens, while not allowing for the substantial growth of any other organism; 2) adding an antimicrobial agent to the medium **without changing the bacterial resistance pattern of the medium**, which could cause non-target organisms to grow or cause non-susceptible target organisms to not grow.

17. Elucidating such a combination of nutrients, selective agents, antibiotics, and other media ingredients presented special difficulties because different combinations of antibiotics and other media ingredients interact with each other to exert differing and often unpredictable effects on cells growing in the medium. In some cases, these interactions will confer increased resistance to certain antibiotics on the part of the target organism(s) or on the part of one or more non-target organisms. **It is generally known in the field of microbiology that organisms susceptible to a particular antibiotic in some media may not be susceptible to the same antibiotic in other media, and that small changes in medium ingredients can effect substantial changes in resistance patterns**. This is sometimes referred to as "antibiotic cross-reactivity." Therefore, development of the antimicrobial susceptibility medium was by no means simply a matter of adding antimicrobial agent to the uropathogenic specific medium. Rather, it involved evaluating what changes in resistance patterns occurred by introduction of the antibiotic.

18. Thus, changing the nutrients in the medium or the combination of antibiotics may change the biological dynamic of the medium and allow organisms to grow that wouldn't grow previously, and vice versa. This would then result in false positives or false negatives. All of these obstacles had to be overcome in arriving at a medium which allows for the growth of the primary gram negative uropathogens but not for the substantial growth of any other organism.

I declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Respectfully submitted,

Chun-Ming Chen

Date: March 22, 2005

IDEXX Laboratories, Inc.
One IDEXX Drive
Westbrook, Maine 04092

# CURRICULUM VITAE

Chun-Ming Chen

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## Date and Place of Birth

June 17, 1957, Tainan, Taiwan.

## Education

1981. B.S. Food Science, Fu-Jen Catholic University, Taipei, Taiwan

1988. M.S. Food Science, University of Florida, Gainesville, FL.

Thesis Advisor: Dr. Cheng-I Wei
Thesis Title:
"Microbiological Assessment and Controlling of Histamine Production in Yellowfin Tuna: Detection and Control of Histamine-producing Bacteria"

1993. Ph.D. Food Science, Rutgers University, NJ.

Thesis Advisor: Dr. Paul H. Tomasek
Thesis Title:
"Purification and Characterization of 3,4-Dihydroxyxanthone Dioxygenase and Gentisate Dioxygenase from *Arthrobacter* sp. strain GFB 100."

## Appointments

| | |
|---|---|
| 1993-Present | Research Scientist<br>Food and Environmental Division<br>IDEXX Laboratories, Inc. Westbrook, ME 04092, U.S.A. |
| 1993 | Postdoctoral Research Fellow<br>Center for Agricultural Molecular Biology<br>Rutgers University |
| 1989-1993 | Graduate Research Assistant<br>Department of Food Science<br>Rutgers University |
| 1985-1988 | Graduate Research Assistant<br>Department of Food Science<br>University of Florida |
| 1983-1985 | Research Associate<br>Graduate Department of Food Science<br>Fu-Jen Catholic University, Taiwan. |

## Professional Affiliation

American Society for Microbiologist (1988-present)
American Water Work Association (1995-present)

## Publications

Chen, C.-M. and C.I. Wei. 1988. Determination of minimum temperature for histamine production by five bacteria. In Proceedings of the 12th Annual Conference of Tropical and Subtropical Fisheries Technological Society of the Americas. 12:365.

Cohen, M.D., C.-M. Chen, and C.I. Wei. 1989. Decreased resistance of *Listeria monocytogenes* in mice following vanadate exposure: effects upon the function of macrophages. Int. J. Immunopharmac. 11:265-292.

Chen, C.-M., C.I. Wei, J.A. Koburger, and M.R. Marshall. 1989. Comparison of four agar media for detection of histamine-producing bacteria in tuna. J. Food Prot. 52:808-813.

Chen, C.-M. 1989. Microbiological assessment and controlling of histamine production in yellowfin tuna: detection and control of histamine-producing bacteria. M.S. thesis. University of Florida.

Wei, C.I., C.-M. Chen, J.A. Koburger, W.S. Otwell, and M.R. Marshall. 1990. Bacterial growth and histamine production on vacuum packaged tuna. J. Food Sci. 55(1):59-63.

Solberg, M., J.J. Buckalew, C.-M. Chen, D.W. Schaffner, K. O'Neill, J. McDowell, L.S. Post, and M. Boderck. 1990. Microbiological Safety Assurance System for Food Service Facilities. Food Technol. Dec. 68-73.

Chen, C.-M. and P.H. Tomasek. 1991. 3,4-Dihydroxyxanthone dioxygenase from *Arthrobacter* sp. strain GFB 100. Appl. Environ. Microbiol. 57:2217-2222.

Chen, C.-M. 1993. Purification and characterization of 3,4-dihydroxyxanthone dioxygenase and gentisate dioxygenase from *Arthrobacter* sp. strain GFB 100. Ph.D. thesis. Rutgers University.

Roff, J.P., C.-M. Chen, and P.H. Tomasek. Purification of an *Arthrobacter* sp. strain GFB 100 4-hydroxy-3-(2'-oxo-3-trans-butenoic acid)-coumarin reductase and identification of its reaction metabolite. (in preparation)

Chen, C.-M. and P.H. Tomasek. Purification and characterization of 3,4-dihydroxyxanthone dioxygenase from xanthone-degrading *Arthrobacter* sp. strain GFB 100 (in preparation)

Chen, C.-M. and P.H. Tomasek. Purificaion and characterization of gentisate dioxygenase from xanthone-degrading *Arthrobacter* sp. strain GFB100. (in preparation)

Chen. C.-M., Haoyi Gu, K. Doherty, and A. Naqui. Evaluation of a defined substrate Enterolert™ system for the detection of enterococci in water. (in preparation)

**Abstracts**

Chen, C-M. and C.-I. Wei. 1987. Determination of minimal temperature for histamine production by five bacteria. 12th Annual Conference of Tropical and Subtropical Fishreies Technological Society of the Americas. Nov. 10. Orlando, FL.

Wei, C.-I., C.-M. Chen, J.A. Koburger, W.S. Otwell, and M.R. Marshall. 1989. A comparison of four agar media for early detection of prolific histamine producing bacteria in tuna samples. The 89th Annual Meeting of the American Society for Microbiology. May 14-18, New Orleans, L.A.

Solberg, M., J.J. Buckalew, C.-M. Chen, and D. W. Schaffner. 1990. Microbial safety assurance system for food service facilities. Food Service Symposium. 51th Annual Meeting, Institute of Food Technologists. June. Anaheim, CA.

Wei, C.I., C-M. Chen. J.A. Koburger, W.S. Otwell, and M.R. Marshall. 1990. Bacterial growth and histamine production on vacuum packaged tuna. The 90th Annual Meeting of the American Society for Microbiology. May 14-18. Anaheim. CA.

Chen. C-M. and P.H. Tomasek. 1990. Initial characterization of 3,4-dihydroxyxanthone dioxygenase from Arthrobacter sp. GFB100. The 90th Annual Meeting of the American Society for Microbiology. May 14-18. Anaheim, CA.

Chen, C-M. and P.H. Tomasek. 1991. *Arthrobacter* 3,4-dihydroxyxanthone dioxygenase: an extradiol ring-fission xanthone catabolic enzyme. Theobald Smith Society Annual Meeting, April 18. Woodbridge, NJ.

Chen. C-M. and P.H. Tomasek. 1991. *Arthrobacter* 3,4-dihydroxyxanthone dioxygenase: purification and characterization. American Society for Microbiology Biotechnology Meeting. New York.

Tomasek, P.H. and C.-M. Chen. 1993. Arthrobacter 3,4-dihydroxyxanthone dioxygenasae: substrate specificity and mechanistic implication for substrate binding. Keystone Symposium for Biodegradation. March 6-12. Lake Taho, CA.

Chen. C-M. and P.H. Tomasek. 1993. Purification and properties of gentisate 1,2-dioxygenae from *Arthrobacter* sp. strain GFB100. The 93rd Annual Meeting of the American Society for Microbiology. May 16-20. Atlanta, GA.

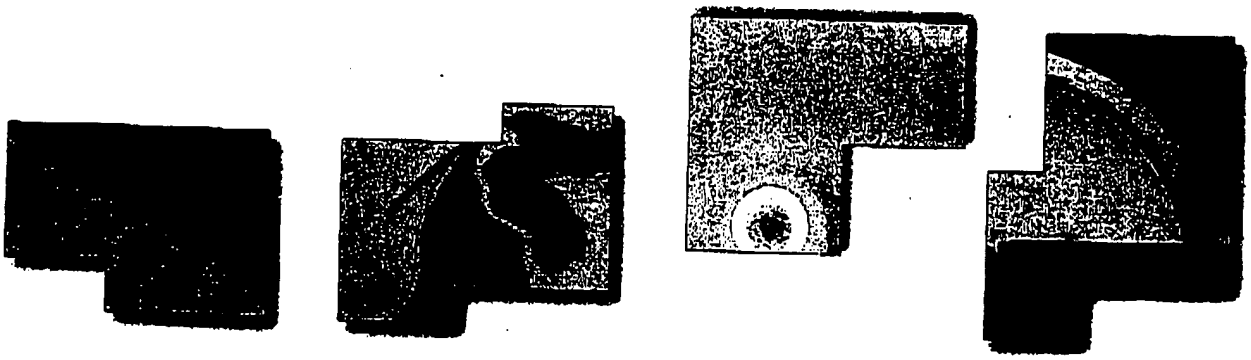
Chen. C-M., P. Coombs, and A. Naqui. 1994. A novel, easy-to-use method for the detection of yeasts in water samples. 41th Annual Conference of Society of Soft Drink Technologists. April 17-20, Albuquerque, New Mexico.

Chen. C-M., K. Doherty, and A. Naqui. 1996. An easy-to-use method for rapid screening of yeast contamination in water samples from soft drink bottling plants. The 96th Annual Meeting of the American Society for Microbiology. May. (Abstract Accepted)

Chen, C.-M., K. Doherty, H. Gu, G. Dichter, and A. Naqui. 1996. Enterolert™ - A rapid method for the detection of Enterococcus spp. in water. The 96th Annual Meeting of the American Society for Microbiology. May. (Abstract Accepted)

# Difco Manual

11th Edition



# MacConkey Media

# Bacto® MacConkey Agar · Bacto MacConkey Agar Base Bacto MacConkey Agar CS · Bacto MacConkey Agar w/o CV Bacto MacConkey Agar w/o Salt

## Intended Use

MacConkey Media are selective and differential plating media mainly used for the detection and isolation of gram-negative organisms from clinical,[1] dairy,[2] food,[3,4] water,[5] pharmaceutical[6] and industrial[7] sources.

**Bacto MacConkey Agar** is used for isolating and differentiating lactose-fermenting from lactose nonfermenting gram-negative enteric bacilli.

**Bacto MacConkey Agar Base** is used with added carbohydrate in differentiating coliforms based on fermentation reactions.

**Bacto MacConkey Agar CS** is used for isolating and differentiating gram-negative enteric bacilli from specimens containing swarming strains of *Proteus*.

**Bacto MacConkey Agar w/o CV** is used for isolating and differentiating enteric microorganisms while permitting growth of staphylococci and enterococci.

**Bacto MacConkey Agar w/o Salt** is used for isolating and differentiating gram-negative bacilli while suppressing the swarming of most *Proteus* species.

## User Quality Control

### Identity Specifications

**MacConkey Agar**

| | |
|---|---|
| Dehydrated Appearance: | Pink to pinkish beige, free-flowing, homogenous. |
| Solution: | 5.0% solution, soluble in distilled or deionized water on boiling; reddish purple, very slightly to slightly opalescent. |
| Prepared Plates: | Pinkish red, slightly opalescent. |
| Reaction of 5.0% Solution at 25°C: | pH 7.1 ± 0.2 |

**MacConkey Agar Base**

| | |
|---|---|
| Dehydrated Appearance: | Pink to pinkish beige, free-flowing, homogenous. |
| Solution: | 4.0% solution, soluble in distilled or deionized water upon boiling; red, very slightly to slightly opalescent without significant precipitate. |
| Prepared Plates: | Red, slightly opalescent without precipitate. |
| Reaction of 4.0% Solution at 25°C: | pH 7.1 ± 0.2 |

**MacConkey Agar CS**

| | |
|---|---|
| Dehydrated Appearance: | Pinkish beige, homogenous, free-flowing. |
| Solution: | 5.0% solution, soluble in distilled or deionized water on boiling; reddish purple in color, slightly opalescent, without significant precipitate. |
| Prepared Plates: | Reddish purple, slightly opalescent, without precipitate. |
| Reaction of 5.0% Solution at 25°C: | pH 7.1 ± 0.2 |

*continued on following page*

## Also Known As

MacConkey Agar is also known as MAC.

## Summary and Explanation

MacConkey Agar is based on the bile salt-neutral red-lactose agar of MacConkey.[8]

The original MacConkey medium was used to differentiate strains of *Salmonella typhosa* from members of the coliform group. Formula modifications improved the growth of *Shigella* and *Salmonella* strains. These modifications included the addition of 0.5% sodium chloride, decreased agar content, and altered bile salts and neutral red concentrations. The formula improvements gave improved differential reactions between these enteric pathogens and the coliform group.

**MacConkey Agar** contains crystal violet and bile salts that inhibit gram-positive organisms and allow gram-negative organisms to grow. Isolated colonies of coliform bacteria are brick red in color and may be surrounded by a zone of precipitated bile. This bile precipitate is due to a local pH drop around the colony due to lactose fermentation. Colonies that do not ferment lactose (such as typhoid, paratyphoid and dysentery bacilli) remain colorless. When lactose non-fermenters grow in proximity to coliform colonies, the surrounding medium appears as cleared areas.

**MacConkey Agar Base** is prepared without added carbohydrates, which permits their addition either individually or in combination. It is recommended that carbohydrates such as sucrose or lactose be added in a concentration of 1% to the basal medium.

**MacConkey CS** ("Controlled Swarming") contains carefully selected raw materials to reduce the swarming of *Proteus* species which could cause difficulty in isolating and enumerating other gram-negative bacilli.

**MacConkey Agar w/o CV** (Crystal Violet) is a differential medium that is less selective than MacConkey Agar. The lack of crystal violet permits the growth of *Staphylococcus* and *Enterococcus*. Staphylococci produce pale pink to red colonies and enterococci produce compact tiny red colonies either on or beneath the surface of the medium.

**MacConkey Agar w/o Salt** is a differential medium that restricts the swarming of *Proteus* species to aid in the detection and isolation of enteric microorganisms. In addition, this medium does not contain crystal violet, allowing *Staphyloccocus* and *Enterococcus* species to grow.

## Principles of the Procedure

Bacto Peptone and Proteose Peptone are sources of nitrogen and other nutrients. Lactose is a fermentable carbohydrate. When lactose is fermented, a local pH drop around the colony causes a color change in the pH indicator (neutral red) and bile precipitation. Bile Salts, Bile Salts No. 3 and Crystal Violet are selective agents that inhibit growth of gram-positive organisms. Bacto Agar is a solidifying agent.

## Formula

### MacConkey Agar

Formula Per Liter

| | |
|---|---|
| Bacto Peptone | 17 g |
| Bacto Proteose Peptone | 3 g |
| Bacto Lactose | 10 g |
| Bacto Bile Salts, No. 3 | 1.5 g |
| Sodium Chloride | 5 g |
| Bacto Agar | 13.5 g |
| Neutral Red | 0.03 g |
| Bacto Crystal Violet | 0.001 g |

Final pH 7.1 ± 0.2 at 25°C

### MacConkey Agar Base

Formula Per Liter

| | |
|---|---|
| Bacto Peptone | 17 g |
| Bacto Proteose Peptone | 3 g |
| Bacto Bile Salts, No. 3 | 1.5 g |
| Sodium Chloride | 5 g |
| Bacto Agar | 13.5 g |
| Neutral Red | 0.03 g |
| Bacto Crystal Violet | 0.001 g |

Final pH 7.1 ± 0.2 at 25°C

### MacConkey Agar CS

Formula Per Liter

| | |
|---|---|
| Bacto Peptone | 17 g |
| Bacto Proteose Peptone | 3 g |
| Bacto Lactose | 10 g |
| Bacto Bile Salts | 5 g |
| Sodium Chloride | 5 g |
| Bacto Agar | 13.5 g |
| Neutral Red | 0.03 g |
| Bacto Crystal Violet | 0.001 g |

Final pH 7.1 ± 0.2 at 25°C

### MacConkey Agar w/o CV

Formula Per Liter

| | |
|---|---|
| Bacto Peptone | 20 g |
| Bacto Lactose | 10 g |
| Bacto Bile Salts | 5 g |
| Sodium Chloride | 5 g |
| Neutral Red | 0.05 g |
| Bacto Agar | 12 g |

Final pH 7.4 ± 0.2 at 25°C

### MacConkey Agar w/o Salt

Formula Per Liter

| | |
|---|---|
| Bacto Peptone | 20 g |
| Bacto Lactose | 10 g |
| Bacto Bile Salts | 5 g |
| Neutral Red | 0.075 g |
| Bacto Agar | 12 g |

Final pH 7.4 ± 0.2 at 25°C

*User Quality Control cont.*

**MacConkey Agar w/o CV**

| | |
|---|---|
| Dehydrated Appearance: | Pinkish beige, free-flowing, homogenous. |
| Solution: | 5.2% solution, soluble in distilled or deionized water upon boiling, reddish orange, clear to very slightly opalescent without significant precipitate. |
| Prepared Plates: | Reddish orange, slightly opalescent without significant precipitate. |
| Reaction of 5.2% Solution at 25°C: | pH 7.4 ± 0.2 |

**MacConkey Agar w/o Salt**

| | |
|---|---|
| Dehydrated Appearance: | Pinkish beige, free-flowing, homogenous. |
| Solution: | 4.7% solution, soluble in distilled or deionized water upon boiling; reddish orange, slightly opalescent. |
| Prepared Plates: | Reddish orange, slightly opalescent. |
| Reaction of 4.7% Solution at 25°C: | pH 7.4 ± 0.2 |

## Cultural Response

Prepare MacConkey media per label directions. Inoculate and incubate at 35 ± 2°C for 18-24 hours.

**MacConkey Agar**

| ORGANISM | ATCC® | INOCULUM CFU | GROWTH | APPEARANCE | BILE PPT. |
|---|---|---|---|---|---|
| *Enterococcus faecalis* | 29212* | 1,000-2,000 | markedly to completely inhibited | – | – |
| *Escherichia coli* | 25922* | 100-1,000 | good | pink | + |
| *Proteus mirabilis* | 12453 | 100-1,000 | good | colorless | – |
| *Salmonella typhimurium* | 14028* | 100-1,000 | good | colorless | – |

*continued on following page*

## Precautions

1. For Laboratory Use.
2. **For MacConkey Agar w/o CV**
   **IRRITANT. IRRITATING TO EYES, RESPIRATORY SYSTEM AND SKIN.** Avoid contact with skin and eyes. Do not breathe dust. Wear suitable protective clothing. Keep container tightly closed.

**MacConkey Agar w/o Salt**

**IRRITANT.** IRRITATING TO EYES, RESPIRATORY SYSTEM AND SKIN. Avoid contact with skin and eyes. Do not breathe dust. Wear suitable protective clothing. Keep container tightly closed.

**FIRST AID:** In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. After contact with skin, wash immediately with plenty of water. If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing is

*User Quality Control cont.*

**MacConkey Agar Base**

| ORGANISM | ATCC® | INOCULUM CFU | GROWTH | APPEARANCE | BILE PPT. |
|---|---|---|---|---|---|
| *Enterococcus faecalis* | 29212* | 1,000-2,000 | markedly to completely inhibited | – | – |
| *Escherichia coli* | 25922* | 100-1,000 | good | w/o lactose: colorless w/lactose: pink | + + |
| *Proteus mirabilis* | 12453 | 100-1,000 | good | colorless | – |
| *Salmonella typhimurium* | 14028* | 100-1,000 | good | colorless | – |

**MacConkey Agar CS**

| ORGANISM | ATCC® | INOCULUM CFU | GROWTH | APPEARANCE | BILE PPT. |
|---|---|---|---|---|---|
| *Enterococcus faecalis* | 29212* | 1,000-2,000 | markedly to completely inhibited | – | – |
| *Escherichia coli* | 25922* | 100-1,000 | good | pink to red | –/+ |
| *Proteus mirabilis* | 12453 | 100-1,000 | good | colorless, swarming markedly to completely inhibited | – |
| *Salmonella typhimurium* | 14028* | 100-1,000 | good | translucent, colorless | – |

**MacConkey Agar w/o CV**

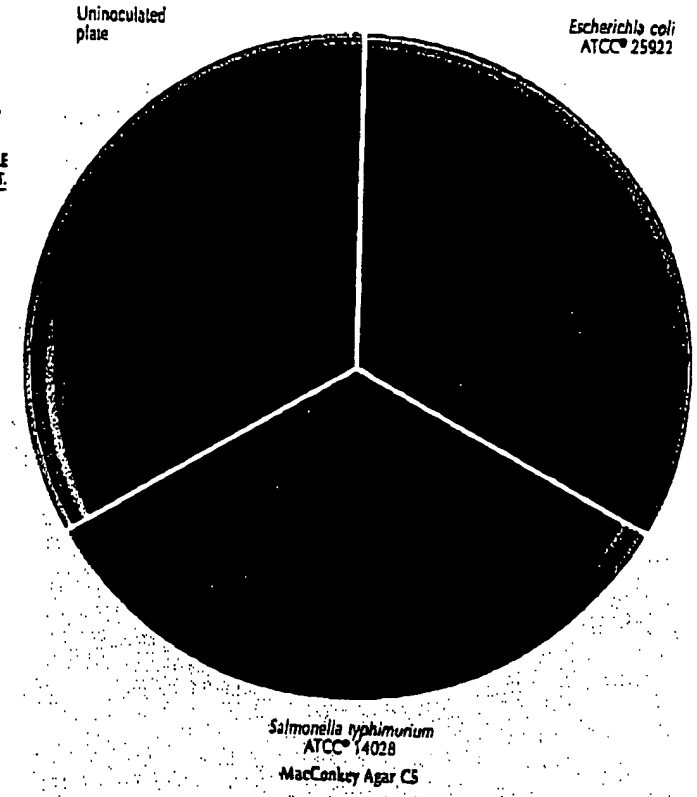
| ORGANISM | ATCC® | INOCULUM CFU | GROWTH | APPEARANCE | BILE PPT. |
|---|---|---|---|---|---|
| *Enterococcus faecalis* | 29212* | 100-1,000 | good | red | – |
| *Escherichia coli* | 25922* | 100-1,000 | good | pink or red | – |
| *Proteus mirabilis* | 12453 | 100-1,000 | good | colorless | – |
| *Salmonella typhimurium* | 14028* | 100-1,000 | good | colorless | – |

**MacConkey Agar w/o Salt**

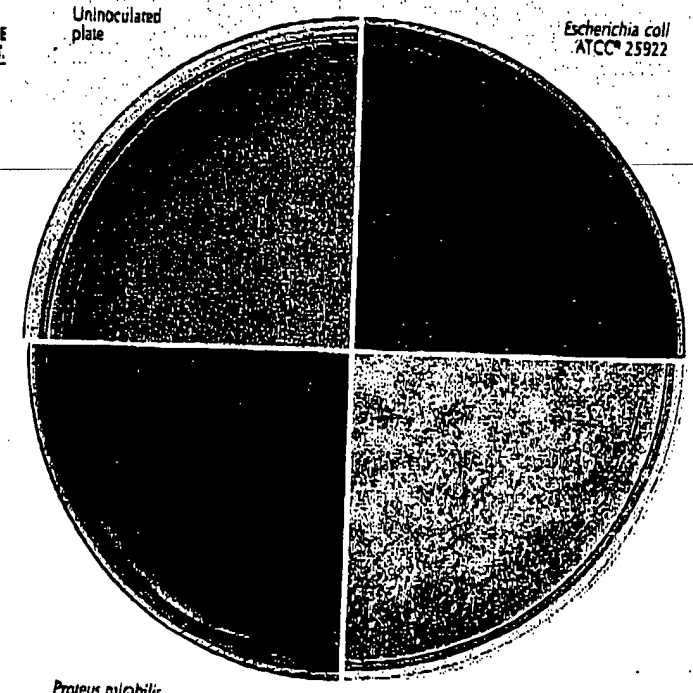
| ORGANISM | ATCC® | INOCULUM CFU | GROWTH | APPEARANCE | BILE PPT. |
|---|---|---|---|---|---|
| *Enterococcus faecalis* | 29212* | 100-1,000 | good | red | – |
| *Escherichia coli* | 25922* | 100-1,000 | good | pink to red | – |
| *Proteus mirabilis* | 12453 | 100-1,000 | good | colorless; swarming markedly to completely inhibited | – |
| *Salmonella typhimurium* | 14028* | 100-1,000 | good | colorless | – |

The cultures listed are the minimum that should be used for performance testing.

*These cultures are available as Bactrol™ Disks and should be used as directed in Bactrol Disks Technical Information.




MacConkey Agar CS




MacConkey Agar w/o CV

difficult, give oxygen. Seek medical advice. If swallowed seek medical advice immediately and show this container or label.

3. Follow proper established laboratory procedure in handling and disposing of infectious materials.

## Storage

Store the dehydrated medium below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

## Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

## Procedure

### Materials Provided

MacConkey Agar
MacConkey Agar Base
MacConkey Agar CS
MacConkey Agar w/o CV
MacConkey Agar w/o Salt

### Materials Required But Not Provided

Glassware
Autoclave
35°C incubator
50°C waterbath (optional)
Carbohydrate (lactose, sucrose, etc.) (optional)

### Method of Preparation

**For MacConkey Agar, MacConkey Agar CS, MacConkey Agar w/o CV or MacConkey Agar w/o Salt:**

1. Suspend the medium in 1 liter distilled or deionized water:

| | |
|---|---|
| MacConkey Agar | 50 grams |
| MacConkey Agar CS | 50 grams |
| MacConkey Agar w/o CV | 52 grams |
| MacConkey Agar w/o Salt | 47 grams |

2. Heat to boiling to dissolve completely. Avoid overheating.
3. Autoclave at 121°C for 15 minutes. The media may be used without autoclave sterilization if the plates are to be inoculated on the day of preparation.
4. Cool to 45-50°C and dispense into sterile Petri dishes.
5. The surface of the medium should be dry when inoculated. Dry the plates for 1-2 hours with the lids slightly ajar.

**For MacConkey Agar Base:**

1. Suspend 40 grams of medium in 1 liter distilled or deionized water.
2. Heat to boiling to dissolve completely. Avoid overheating.
3. Add 10 grams lactose or other desired carbohydrate before or after sterilization, depending on heat lability.
4. Autoclave at 121°C for 15 minutes. The media may be used without autoclave sterilization if the plates are to be inoculated on the day of preparation. In this case, boiling the medium gently for 5 minutes is sufficient.
5. Cool to 45-50°C and dispense into sterile Petri dishes.
6. The surface of the medium should be dry when inoculated. Dry the plates for 1-2 hours with the lids slightly ajar.

### Specimen Collection and Preparation

For a complete discussion on the isolation and identification of enteric organisms consult the appropriate references.

### Test Procedure

For procedures on the isolation and identification of enteric organisms consult the appropriate references.

### Results

Lactose-fermenting organisms grow as pink to brick-red colonies with or with out a zone of precipitated bile. Non-lactose fermenting organisms grow as colorless or clear colonies.

Swarming by *Proteus* spp. is reduced on MacConkey Agar CS and MacConkey Agar w/o Salt.

On MacConkey Agar w/o CV and MacConkey Agar w/o Salt, staphylococci produce pale pink to red colonies and enterococci produce tiny red colonies; these organisms are inhibited on MacConkey Agar and MacConkey Agar CS.

## Limitations of the Procedure

1. Although MacConkey media are selective primarily for gram-negative enteric bacilli, biochemical and, if indicated, serological testing using pure cultures are recommended for complete identification. Consult appropriate references for further information.[1,3]
2. Due to the selective properties of MacConkey Agar CS, some strains of gram-negative enteric bacilli may be encountered that fail to grow or grow poorly on this medium. Some strains of gram-positive organisms may be encountered that are not inhibited or only partially inhibited on this medium; some strains of enterococci may grow on MacConkey Agar CS after prolonged incubation.
3. Incubation of MacConkey Agar plates under increased $CO_2$ has been reported to reduce the growth and recovery of a number of strains of gram-negative bacilli.[9]
4. For optimal performance, plates prepared from MacConkey Agar CS should be incubated under aerobic conditions.

## References

1. **Gray, L. D.** 1995. *Escherichia, Salmonella, Shigella and Yersinia*, p. 450-456. *In* P. R. Murray, E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H Yolken (ed.), Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.
2. **Flowers, R. S., W. Andrews, C. W. Donnelly, and E. Koenig (H.M. Wehr, Tech. Comm.).** 1992. Pathogens in milk and milk products, p. 103-212. *In* R. T. Marshall, (ed.). Standard methods for the examination of dairy products. 16th ed., American Public Health Association, Washington, D.C.

3. **Hitchins, A. D., P. A. Hartman, and E. C. D. Todd.** 1992. Coliforms-*Escherichia coli* and its Toxins, p. 325-369. *In* C. Vanderzant, and D. F. Splittstoesser (ed.), Compendium of methods for the microbiological examination of foods, 3rd ed. American Public Health Association, Washington. D.C.
4. **Food and Drug Administration.** 1995. Bacteriological analytical manual, 8th ed. AOAC International. Gaithersburg, MD.
5. **Eaton, A. D., L. S. Clesceri, and A.E. Greenberg (ed.).** 1995. Standard methods for the examination of water and wastewater, 19th ed. American Public Health Association, Washington, D.C.
6. **United States Pharmacopeial Convention, Inc.** 1995. The United States pharmacopeia, 23rd ed. The United States Pharmacopeial Convention. Rockville, MD.
7. **Association of Official Analytical Chemists.** 1995. Official methods of analysis of AOAC International. 16th ed. AOAC International, Arlington, VA.
8. **MacConkey, A.** 1905. Lactose-fermenting bacteria in feces. J. Hyg. 5:333-379.
9. **Mazura-Reetz, G. T. Neblett, and J. M. Galperin.** 1979. MacConkey Agar: $CO_2$ vs. ambient incubation. Abst. Ann. Mtg. American Society for Microbiology. C179.

## Packaging

| | | |
|---|---|---|
| MacConkey Agar | 100 g | 0075-15-3 |
| | 500 g | 0075-17-1 |
| | 2 kg | 0075-07-3 |
| | 10 kg | 0075-08-2 |
| MacConkey Agar Base | 500 g | 0818-17-3 |
| MacConkey Agar CS | 500 g | 1818-17-1 |
| | 2 kg | 1818-07-3 |
| | 10 kg | 1818-08-2 |
| MacConkey Agar w/o CV | 500 g | 0470-17-2 |
| MacConkey Agar w/o Salt | 500 g | 0331-17-1 |
| | 10 kg | 0331-08-2 |

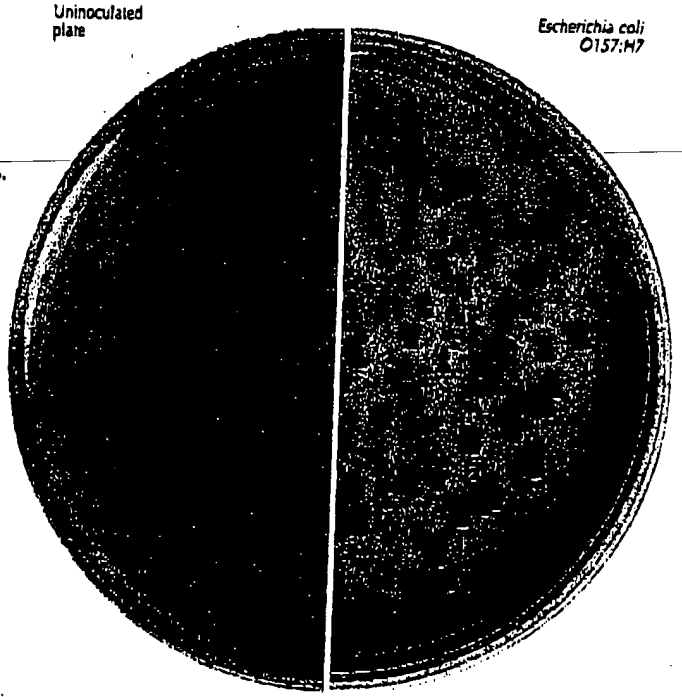
# Bacto® MacConkey Sorbitol Agar

## Intended Use

Bacto MacConkey Sorbitol Agar is used for isolating and differentiating enteropathogenic *Escherichia coli* serotypes.

## Summary and Explanation

The original MacConkey medium was used to differentiate strains of *Salmonella typhosa* from members of the coliform group. Formula modifications used in MacConkey Agar improved the growth of *Shigella* and *Salmonella* strains as well as the differential reactions between these enteric pathogens and the coliform group. The modifications included addition of 0.5% sodium chloride, decreased agar content, and altered bile salts and neutral red concentrations.




## User Quality Control

### Identity Specifications

| | |
|---|---|
| Dehydrated Appearance: | pinkish-beige, free-flowing, homogeneous. |
| Solution: | 5.0% solution, soluble in distilled or deionized water on boiling; reddish purple, very slightly to slightly opalescent. |
| Prepared Medium: | reddish-purple, slightly opalescent. |
| Reaction of 5.0% Solution at 25°C: | pH 7.1 ± 0.2 at 25°C |

### Cultural Response

Prepare MacConkey Sorbitol Agar per label directions. Inoculate plates and incubate at 35 ± 2°C for 18-24 hours.

| ORGANISM | ATCC* | CFU | INOCULUM RECOVERY | COLONY COLOR | BILE PPT |
|---|---|---|---|---|---|
| *Enterococcus faecalis* | 29212* | 1,000-2,000 | markedly inhibited | – | – |
| *Escherichia coli* 0157:H7 | – | 100-1,000 | good | colorless | – |
| *Escherichia coli* | 25922* | 100-1,000 | good | pink-red | + |

The cultures listed are the minimum that should be used for performance testing.
*These cultures are available as Bactrol™ Disks and should be used as directed in Bactrol Disks Technical Information.

4. Prepare plates such as Violet Red Bile Agar for streaking. To ensure recovery of dextrose fermenters, add 1% dextrose before boiling.
5. Streak a loopful of the enrichment culture onto the prepared plates.
6. Incubate the plates for 18-24 hours at 35-37°C. Examine for the presence of coliforms which appear pink to purplish-red on Violet Red Bile Agar. The color of coliform colonies may vary if a different medium is used.

For a complete discussion on *Enterobacteriaceae* in food testing, refer to procedures in Standard Methods.[3,4]

### Results

Acid production causes the color of EE Broth Mossel to become yellow. A negative reaction results in no color change and the medium remains green.

## Limitations of the Procedure

1. Since the nutritional requirements of organisms vary, some strains may be encountered that fail to grow or grow poorly on this medium.

## References


1. **Mossel, D. A. A., M. Vissar, and A. M. R. Cornellisen.** 1963. The examination of foods for *Enterobacteriaceae* using a test of the type generally adopted for the detection of salmonellae. J. Appl. Bacteriol. 26:444-452.
2. **Hartman, P. A., and S. A. Minnich.** 1981. Automation for rapid identification of *Salmonella* in foods. J. Food Prot. 44:385-386.
3. **Association of Official Analytical Chemists.** 1995. Bacteriological analytical manual, 8th ed. AOAC International, Gaithersburg, MD.
4. **Vanderzant, C., and D. F. Splittstoesser (ed.).** 1992. Compendium of methods for the microbiological examination of food, 3rd ed. American Public Health Association, Washington, D.C.


## Packaging

| | | |
|---|---|---|
| EE Broth Mossel | 500 g | 0566-17 |
| | 10 kg | 0566-08 |

# Bacto® EMB Agar

## Intended Use

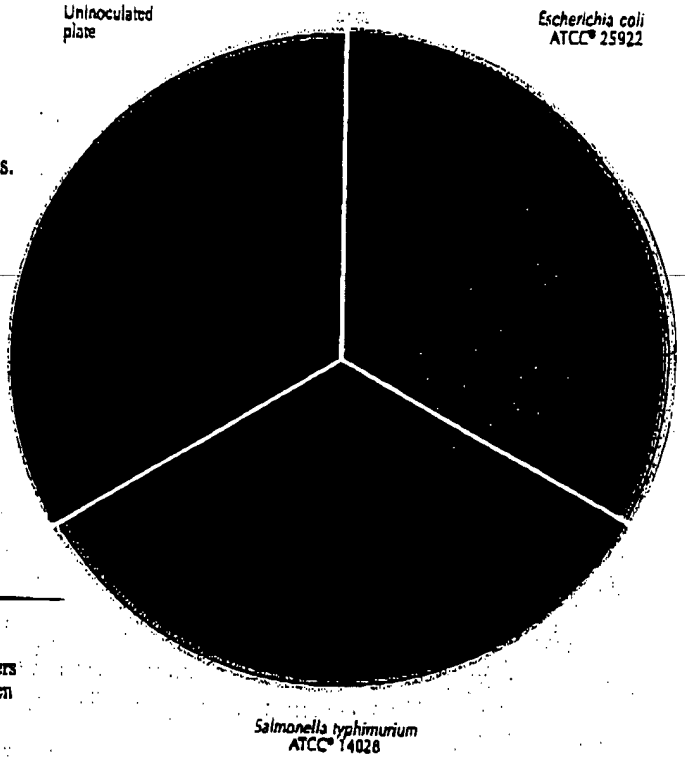
Bacto EMB Agar is used for isolating and differentiating gram-negative enteric bacilli.

## Also Known As

EMB Agar is also known as Eosin Methylene Blue Agar

## Summary and Explanation

The original Eosin Methylene Blue Agar was the formulation of Holt-Harris and Teague.[1] The use of eosin and methylene blue as indicators gave sharp and distinct differentiation between colonies of lactose fermenting and nonfermenting organisms. Sucrose was included in the medium to detect members of the coliform group that fermented sucrose more readily than lactose. Lactose-positive colonies were black




## User Quality Control

### Identity Specifications

| | |
|---|---|
| Dehydrated Appearance: | Pinkish purple, free flowing, homogeneous. |
| Solution: | 3.6% solution, soluble in distilled or deionized water on boiling. Solution is green with orange cast, opalescent with a uniform flocculent precipitate. |
| Prepared Plates: | Purple with a greenish-orange cast, opalescent, may have a fine precipitate. |
| Reaction of 3.6% Solution at 25°C: | pH 7.2 ± 0.2 |

### Cultural Response

Prepare EMB Agar per label directions. Inoculate and incubate at 35 ± 2°C for 18-24 hours.

| ORGANISM | ATCC® | INOCULUM CFU | GROWTH | APPEARANCE |
|---|---|---|---|---|
| *Enterococcus faecalis* | 29212* | 1,000 | partial inhibition | colorless |
| *Escherichia coli* | 25922* | 100-1,000 | good | blue-black w/dark centers and green metallic sheen |
| *Salmonella typhimurium* | 14028* | 100-1,000 | good | colorless to amber |

The cultures listed are the minimum that should be used for performance testing.
*These cultures are available as Bactrol™ Disks and should be used as directed in Bactrol Disks Technical Information.

or possessed dark centers with transparent, colorless peripheries. Lactose- or sucrose-negative colonies were colorless. The Eosin Methylene Blue Agar of Holt-Harris and Teague had definite advantages over the Fuchsin Sulfite Agar of Endo. The EMB Agar formulation was more sensitive, more accurate, more stable, and gave an earlier differentiation between the lactose fermenters and lactose and sucrose nonfermenters.

Two years after Holt-Harris and Teague had introduced their new medium, Levine[2] described an Eosin Methylene Blue Agar for differentiating fecal and nonfecal coliforms. Levine's medium differentiated salmonellae and other lactose nonfermenters from the coliform organisms.

EMB Agar is a combination of the Levine and the Holt-Harris and Teague formulae. EMB Agar is selective due to the presence of inhibitors and differential based on the ability of some organisms to ferment carbohydrates with the absorption of eosin and methylene blue.

EMB Agar is recommended for use in examining clinical specimens for enteric pathogens.[3,4,5] The medium enables the isolation and differentiation of gram-negative enteric bacilli.

## Principles of the Procedure

Peptone is a source of nitrogen and other nutrients in the formulation. Eosin and methylene blue are dyes which combine to form a precipitate at an acid pH. The dyes act both as pH indicators and inhibitors. Gram-positive bacteria are partially inhibited on the medium. Lactose and Sucrose are fermentable carbohydrates. Phosphate acts as a buffer. Bacto Agar is a solidifying agent.

## Formula

**EMB Agar**

Formula Per Liter

| | |
|---|---|
| Bacto Peptone | 10 g |
| Bacto Lactose | 5 g |
| Bacto Sucrose | 5 g |
| Dipotassium Phosphate | 2 g |
| Bacto Agar | 13.5 g |
| Eosin Y | 0.4 g |
| Methylene Blue | 0.065 g |

Final pH 7.2 ± 0.2 at 25°C

## Precautions

1. For Laboratory Use.
2. Follow proper established laboratory procedure in handling and disposing of infectious materials.

## Storage

Store the dehydrated medium below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed. Store prepared plates at 2-8°C.

## Expiration Date

Expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

## Procedure

### Materials Provided

EMB Agar

### Materials Required But Not Provided

Flasks with closures
Distilled or deionized water
Bunsen burner or magnetic hot plate
Autoclave
Waterbath (45-50)°C
Petri dishes
Incubator (35°C)

### Method of Preparation

1. Suspend 36 grams in 1 liter distilled or deionized water.
2. Heat to boiling to dissolve completely.
3. Autoclave at 121°C for 15 minutes. Avoid overheating.
4. Cool to 45-50°C in a waterbath.
5. Dispense into sterile Petri dishes. Evenly disperse the precipitate when dispensing.

### Specimen Collection and Preparation

1. Collect specimens in sterile containers or with sterile swabs and transport immediately to the laboratory following recommended guidelines.[3,4,5]
2. For specific information about specimen preparation and inoculation of clinical specimens, consult appropriate references.[3,4,5]

### Test Procedure

For isolation of enteric pathogens from clinical specimens, inoculate fecal specimens and rectal swabs onto a small area of one quadrant of the EMB Agar plate and streak for isolation. This will permit development of discrete colonies. Incubate plates at 35°C. Examine plates at 24 hours and again at 48 hours for colonies with characteristic morphologies associated with potential pathogens.

### Results

*Salmonella* and *Shigella* colonies are translucent and amber colored or colorless. Coliforms that use lactose and/or sucrose produce blue-black colonies with dark centers and greenish metallic sheen. Other coliforms such as *Enterobacter* form mucoid, pink colonies. Strains of *Enterococcus faecalis* are partially inhibited on this medium and appear as colorless colonies.

## Limitations of the Procedure

1. EMB Agar is only moderately inhibitory. Some staphylococci, streptococci and yeast may grow. They will appear as small, pinpoint colonies. Gram-negative nonfermenting bacilli may grow and appear as non-lactose fermenters. Biochemical tests are necessary for further identification to genus or species.[6]
2. Some strains of *Salmonella* and *Shigella* may not grow on EMB Agar.[6] It is recommended that a nonselective, differential medium (MacConkey Agar or Hektoen Enteric Agar) and a selective medium (Bismuth Sulfite Agar, SS Agar or Desoxycholate Citrate Agar) be run in parallel with EMB Agar.
3. Sterilization reduces the methylene blue, leaving the medium orange in color. The normal purple color of the medium may be restored by gentle mixing. If the reduced medium is not shaken to oxidize the methylene blue, a dark zone beginning at the top and extending downward through the medium will gradually appear. The sterilized medium normally contains a flocculent precipitate which should not be removed. By cooling to 50°C and gently

mixing the medium before pouring it into plates, the flocculation will be finely dispersed.

4. Greenish metallic sheen is **not** always present. The presence of the greenish metallic sheen is **not** diagnostic for *E. coli*.[6]
5. Store and incubate EMB Agar plates in the dark. Visible light can alter the ability of the medium to support microbial growth, especially of *Proteus* spp.[7]

## References


1. **Holt-Harris, J. E., and O. Teague.** 1916. A new culture medium for the isolation of *Bacillus typhosa* from stools. J. Infect. Dis. 18:596-600.
2. **Levine, M. M.** 1918. Differentiation of *E. coli* and *B. aerogenes* on a simplified Eosin-Methylene Blue Agar. J. Infect. Dis. 23:43.
3. **Gray, L. D.** 1995. *Escherichia, Salmonella, Shigella and Yersinia*, p. 450-456. *In* P. R. Murray, E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken (ed.), Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.
4. **Pezzlo, M. (ed.).** 1992. Aerobic bacteriology, p. 1.0.1-1.20.47. *In* H. D. Isenberg, (ed.), Clinical microbiology procedures handbook, vol. 1. American Society for Microbiology, Washington, D.C.
5. **Baron, E. J., L. R. Peterson, and S. M. Finegold.** 1994. Bailey & Scott's diagnostic microbiology, 9th ed. Mosby-Year Book, Inc., St. Louis, MO.
6. **MacFaddin, J. F.** 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1. Williams & Wilkins, Baltimore, MD.
7. **Girolami, R. L., and J. M. Stamm.** 1976. Inhibitory effect of light on growth-supporting properties of Eosin Methylene Blue Agar. Appl. Environ. Microbiol. 31:141.


## Packaging

| | | |
|---|---|---|
| EMB Agar | 100 g | 0076-15 |
| | 500 g | 0076-17 |
| | 2 kg | 0076-07 |
| | 10 kg | 0076-08 |

# Bacto® EVA Broth

## Intended Use

Bacto EVA Broth is used for detecting and confirming enterococci in water and other specimens as an indication of fecal contamination.

## Also Known As

EVA Broth is also known as Ethyl Violet Azide Broth.

## Summary and Explanation

The presence of enterococci in water and other specimens indicates fecal contamination. Mallmann and Seligmann[1] compared various enrichment media for detecting fecal streptococci and found that Azide Dextrose Broth presumptively identified the streptococci. However, because gram-positive bacteria other than enterococci grow in the medium, confirmation is necessary. Litsky et al.[2] studied various dyes and selective agents and formulated a medium using ethyl violet and sodium azide as selective agents. The medium known as Ethyl Violet Azide (EVA) Broth is specific for enterococci. In conjunction with Azide Dextrose Broth, EVA Broth is used to confirm the presence of enterococci.

## Principles of the Procedure

EVA Broth contains Tryptose as a source of carbon, nitrogen, vitamins and minerals. Dextrose is the carbohydrate. Sodium Azide and

## User Quality Control

### Identity Specifications

Dehydrated Appearance: Light beige, free-flowing, homogeneous.

Solution: 3.58% solution, soluble in distilled or deionized water. Solution is light amber, clear to very slightly opalescent.

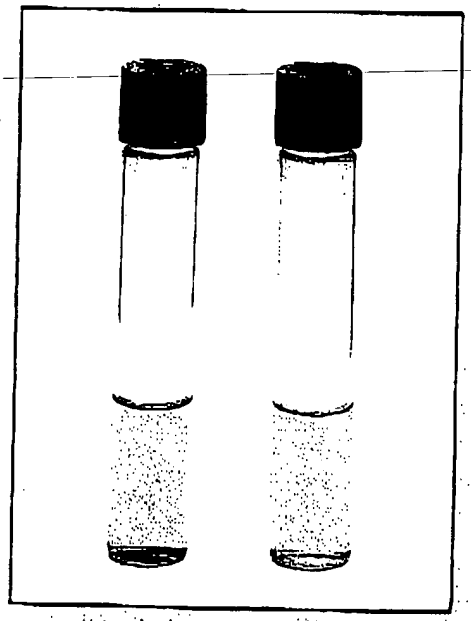
Reaction of 3.58% Solution at 25°C: pH 7.0 ± 0.2

### Cultural Response

Prepare EVA Broth per label directions. Inoculate and incubate the tubes at 35 ± 2°C for 18-48 hours.

| ORGANISM | ATCC® | INOCULUM CFU | GROWTH |
|---|---|---|---|
| *Enterococcus faecalis* | 19433* | 100-1,000 | good |
| *Enterococcus faecalis* | 29212* | 100-1,000 | good |
| *Escherichia coli* | 25922* | 1,000-2,000 | inhibited |

The cultures listed are the minimum that should be used for performance testing.
*These cultures are available as Bactrol™ Disks and should be used as directed in Bactrol Disks Technical Information.



Uninoculated tube — *Enterococcus faecalis* ATCC® 29212

7th EDITION

# *Manual of* CLINICAL MICROBIOLOGY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Fred C. Tenover's role as an editor of this book was carried out completely in his private capacity and his contribution as an editor in no way reflects official support or endorsement by the Centers for Disease Control and Prevention.

# *Enterobacteriaceae*: Introduction and Identification

J. J. FARMER III

# 27

## INTRODUCTION

In the fifth edition of this Manual in 1991, Farmer and Kelly commented that it was becoming more difficult to cover the family *Enterobacteriaceae* in a single chapter. The family includes the plague bacillus *Yersinia pestis*; the typhoid bacillus *Salmonella* serotype Typhi (*Salmonella typhi*); four genera with species that often cause diarrhea and other intestinal infections; seven species that frequently cause nosocomial infections; many other organisms that occasionally cause human or animal infections; dozens of species that occasionally occur in human clinical specimens; and many other species that do not occur in human clinical specimens but can be confused with those that do. In the fifth edition, all these organisms were covered in a single chapter on *Enterobacteriaceae*, divided into six parts. In the sixth edition, the material on *Enterobacteriaceae* was divided among three chapters: an introduction to the family that described the overall plan for isolation and identification; a chapter that covered *Salmonella*, *Shigella*, *Escherichia coli*, and *Yersinia*, the four genera that comprise the enteric pathogens; and a chapter that covered the remaining genera and species in the family. In the current edition further subdivision has occurred and there are now four chapters on *Enterobacteriaceae*: chapters 27 through 30.

Because of space limitations, many topics in the present chapter are discussed briefly and only a few primary literature citations are given. Several books, reviews, and chapters are recommended for more detailed information (4, 12, 13, 22, 27, 33, 39, 71).

## NOMENCLATURE AND CLASSIFICATION

The nomenclature and classification of the genera, species, subspecies, biogroups, and serotypes of the *Enterobacteriaceae* have always been topics for hot debate and differing opinions (12, 13, 22, 27, 33, 69, 71). Until recently, genera and species were defined by biochemical and antigenic analysis. Newer techniques such as nucleic acid hybridization and nucleic acid sequencing that measure evolutionary distance (see chapter 14) have made it possible to determine the evolutionary relationships of organisms in the family (13, 33). The use of DNA-DNA hybridization has led to the discovery of many new species and has resulted in the reclassification of some of the older ones (12, 13, 33).

This chapter includes the different names and classifications that clinical microbiologists are likely to encounter in the scientific literature and in material accompanying commercial products. The nomenclature and classification given in Table 1 are a compromise based on all available evidence and include most of the genera, species, subspecies, biogroups, and unnamed Enteric Groups included in the family. If two names are widely used for the same organism, both are given in this chapter with one in parentheses. Most of the "nonclinical" organisms in the family are also included because there is a possibility that they will be isolated from a human clinical specimen some day (33, 36).

Most of the newly described organisms in Table 1 are only very rarely found in clinical specimens (33); most clinically significant isolates belong to 20 to 25 species that have been well known for many years (27). This is illustrated by the lists of genera that most often cause bacteremia (Table 2), nosocomial infections (Table 3), and infections of the gastrointestinal tract (Table 4). The original citations for many of the newer genera (1, 9, 10, 35, 41, 43, 45, 46, 57–60, 63, 77, 88, 89) and species (2, 3, 7, 8, 14–19, 21, 23, 25, 28–31, 34, 36, 38, 40, 42, 44, 47–50, 52, 54–56, 61, 62, 64–66, 70, 73, 75, 77–79, 85, 90, 92) are given in the References section.

### New Species

Several new species have been described since the sixth edition was published in 1995. These include *Buttiauxella ferragutiae* (77), *Buttiauxella gaviniae* (77), *Buttiauxella brennerae* (77), *Buttiauxella izardii* (77), *Buttiauxella noackiae** (77), *Buttiauxella warmboldiae* (77), *Citrobacter rodentium* (formerly *Citrobacter* species 9) (16), *Enterobacter kobei** (70), *Enterobacter pyrinus* (21), *Kluyvera cochleae* (77), and *Kluyvera georgiana** (77) (those found in human clinical specimens are indicated by an asterisk). These new species have been added to Table 1, except for *Enterobacter kobei* (reference strains have been unavailable). All newly described species of *Enterobacteriaceae* will eventually be studied and added.

### The Expanding Number of *Enterobacteriaceae* Species

How many species of *Enterobacteriaceae* are there? There are probably many hundreds, if not thousands. This is becoming more apparent as methods such as DNA-DNA hybridization and 16S rRNA sequencing are being used routinely to study

**TABLE 1** Biochemical reactions of the named species, biogroups, and Enteric Groups of the family *Enterobacteriaceae*[a]

| Organism | Indole production | Methyl red | Voges-Proskauer | Citrate (Simmons) | Hydrogen sulfide (TSI) | Urea hydrolysis | Phenylalanine deaminase | Lysine decarboxylase | Arginine dihydrolase | Ornithine decarboxylase | Motility (36°C) | Gelatin hydrolysis (22°C) | Growth in KCN | Malonate utilization | D-Glucose, acid | D-Glucose, gas | Lactose fermentation | Sucrose fermentation | D-Mannitol fermentation | Dulcitol fermentation | Salicin fermentation | Adonitol fermentation | *myo*-Inositol fermentation | D-Sorbitol fermentation | L-Arabinose fermentation | Raffinose fermentation | L-Rhamnose fermentation | Maltose fermentation | D-Xylose fermentation | Trehalose fermentation | Cellobiose fermentation | alpha-Methyl-D-glucoside fermentation | Erythritol fermentation | Esculin hydrolysis | Melibiose fermentation | D-Arabitol fermentation | Glycerol fermentation | Mucate fermentation | Tartrate, Jordan's | Acetate utilization | Lipase (corn oil) | DNase at 25°C | Nitrate → Nitrite | Oxidase, Kovacs | ONPG test | Yellow pigment | D-Mannose fermentation |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| ***Budvicia*** | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| *B. aquatica*[b] | 0 | 93 | 0 | 0 | 80 | 33 | 0 | 0 | 0 | 0 | 27 | 0 | 0 | 0 | 100 | 53 | 87 | 0 | 60 | 0 | 0 | 0 | 0 | 0 | 80 | 0 | 100 | 0 | 93 | 0 | 0 | 0 | 0 | 0 | 0 | 27 | 0 | 20 | 27 | 0 | 0 | 0 | 100 | 0 | 93 | 0 | 0 |
| ***Buttiauxella*** | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| *B. agrestis* | 0 | 100 | 0 | 100 | 0 | 0 | 0 | 0 | 0 | 100 | 100 | 0 | 80 | 60 | 100 | 100 | 100 | 0 | 100 | 0 | 100 | 0 | 0 | 0 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 0 | 0 | 100 | 100 | 0 | 60 | 100 | 60 | 0 | 0 | 0 | 100 | 0 | 100 | 0 | 100 |
| *B. brennerae* | 0 | 100 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 33 | 100 | 0 | 100 | 100 | 100 | 100 | 67 | 0 | 100 | 0 | 100 | 67 | 0 | 0 | 100 | 100 | 33 | 100 | 100 | 100 | 100 | 100 | 0 | 0 | 100 | 100 | 67 | 67 | 67 | 0 | 0 | 0 | 0 | 100 | 0 | 100 | 0 | 100 |
| *B. ferragutiae* | 0 | 100 | 0 | 0 | 0 | 0 | 0 | 100 | 0 | 80 | 60 | 0 | 40 | 0 | 100 | 100 | 0 | 0 | 100 | 0 | 100 | 0 | 0 | 100 | 100 | 0 | 100 | 100 | 100 | 100 | 100 | 40 | 0 | 100 | 0 | 0 | 0 | 60 | 0 | 0 | 0 | 0 | 0 | 100 | 0 | 100 | 0 | 100 |
| *B. gaviniae* | 0 | 100 | 0 | 20 | 0 | 0 | 0 | 0 | 20 | 0 | 80 | 0 | 60 | 100 | 100 | 40 | 60 | 0 | 100 | 0 | 100 | 100 | 0 | 0 | 100 | 0 | 100 | 60 | 100 | 100 | 100 | 0 | 0 | 100 | 0 | 80 | 0 | 80 | 40 | 0 | 0 | 0 | 0 | 100 | 0 | 100 | 0 | 100 |
| *B. izardii* | 0 | 100 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 100 | 100 | 0 | 67 | 100 | 100 | 100 | 100 | 0 | 100 | 0 | 100 | 0 | 0 | 0 | 100 | 33 | 100 | 100 | 100 | 100 | 100 | 0 | 0 | 100 | 67 | 0 | 33 | 100 | 67 | 0 | 0 | 0 | 0 | 100 | 0 | 100 | 0 | 100 |
| *B. noacklae*[a] | 33 | 100 | 0 | 33 | 0 | 0 | 100 | 0 | 67 | 0 | 100 | 0 | 100 | 100 | 100 | 100 | 0 | 0 | 100 | 0 | 100 | 0 | 0 | 0 | 100 | 0 | 100 | 100 | 100 | 100 | 100 | 33 | 0 | 100 | 0 | 0 | 0 | 100 | 100 | 0 | 0 | 0 | 0 | 100 | 0 | 100 | 0 | 100 |
| *B. warmboldiae* | 0 | 100 | 0 | 33 | 0 | 0 | 100 | 0 | 0 | 0 | 100 | 0 | 33 | 100 | 100 | 100 | 0 | 0 | 100 | 0 | 100 | 0 | 67 | 0 | 100 | 0 | 100 | 100 | 100 | 100 | 100 | 0 | 0 | 100 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 100 | 0 | 100 | 0 | 100 |
| ***Cedecea*** | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| *C. davisae*[a] | 0 | 100 | 50 | 95 | 0 | 0 | 0 | 0 | 50 | 95 | 95 | 0 | 88 | 91 | 100 | 70 | 19 | 100 | 100 | 0 | 99 | 0 | 0 | 0 | 0 | 10 | 0 | 100 | 100 | 100 | 100 | 5 | 0 | 45 | 0 | 100 | 0 | 0 | 0 | 0 | 91 | 0 | 100 | 0 | 90 | 0 | 100 |
| *C. lapagei*[a] | 0 | 40 | 80 | 99 | 0 | 0 | 0 | 0 | 80 | 0 | 80 | 0 | 100 | 99 | 100 | 100 | 60 | 0 | 100 | 0 | 100 | 0 | 0 | 0 | 0 | 0 | 0 | 100 | 0 | 100 | 100 | 0 | 0 | 100 | 0 | 100 | 0 | 0 | 0 | 60 | 100 | 0 | 100 | 0 | 99 | 0 | 100 |
| *C. neteri*[a] | 0 | 100 | 50 | 100 | 0 | 0 | 0 | 0 | 100 | 0 | 100 | 0 | 65 | 100 | 100 | 100 | 35 | 100 | 100 | 0 | 100 | 0 | 0 | 0 | 100 | 0 | 0 | 100 | 100 | 100 | 100 | 0 | 0 | 100 | 0 | 100 | 0 | 0 | 0 | 0 | 100 | 0 | 100 | 0 | 100 | 0 | 100 |
| *Cedecea species 3*[a] | 0 | 100 | 50 | 100 | 0 | 0 | 0 | 0 | 100 | 0 | 100 | 0 | 100 | 0 | 100 | 100 | 0 | 50 | 100 | 0 | 100 | 0 | 0 | 0 | 0 | 100 | 0 | 100 | 100 | 100 | 100 | 50 | 0 | 100 | 100 | 100 | 0 | 0 | 0 | 50 | 100 | 0 | 100 | 0 | 100 | 0 | 100 |
| *Cedecea species 5*[a] | 0 | 100 | 50 | 100 | 0 | 0 | 0 | 0 | 50 | 50 | 100 | 0 | 100 | 0 | 100 | 100 | 0 | 100 | 100 | 0 | 100 | 0 | 0 | 100 | 0 | 100 | 0 | 100 | 100 | 100 | 100 | 0 | 0 | 100 | 100 | 100 | 0 | 0 | 0 | 50 | 50 | 0 | 100 | 0 | 100 | 0 | 100 |
| ***Citrobacter*** | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| *C. freundii*[a] | 33 | 100 | 0 | 78 | 78 | 44 | 0 | 0 | 67 | 0 | 89 | 0 | 89 | 11 | 100 | 89 | 78 | 89 | 100 | 11 | 0 | 0 | 0 | 100 | 100 | 44 | 100 | 100 | 89 | 100 | 44 | 11 | 0 | 0 | 100 | 0 | 100 | 100 | 100 | 44 | 0 | 0 | 100 | 0 | 89 | 0 | 100 |
| *C. diversus (koseri)*[a] | 99 | 100 | 0 | 99 | 0 | 75 | 0 | 0 | 80 | 99 | 95 | 0 | 0 | 95 | 100 | 98 | 50 | 40 | 99 | 40 | 15 | 99 | 0 | 99 | 99 | 0 | 99 | 100 | 100 | 100 | 99 | 40 | 0 | 1 | 0 | 98 | 99 | 95 | 90 | 75 | 0 | 0 | 100 | 0 | 99 | 0 | 100 |
| *C. amalonaticus*[a] | 100 | 100 | 0 | 95 | 5 | 85 | 0 | 0 | 85 | 95 | 95 | 0 | 99 | 1 | 100 | 97 | 35 | 9 | 100 | 1 | 30 | 0 | 0 | 99 | 99 | 5 | 100 | 99 | 99 | 100 | 100 | 2 | 0 | 5 | 0 | 0 | 60 | 96 | 96 | 86 | 0 | 0 | 99 | 0 | 97 | 0 | 100 |
| *C. farmeri*[a] | 100 | 100 | 0 | 10 | 0 | 59 | 0 | 0 | 85 | 100 | 97 | 0 | 93 | 0 | 100 | 96 | 15 | 100 | 100 | 2 | 9 | 0 | 0 | 98 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 75 | 0 | 0 | 100 | 0 | 65 | 100 | 93 | 80 | 0 | 0 | 100 | 0 | 100 | 0 | 100 |
| *C. youngae*[a] | 15 | 100 | 0 | 75 | 65 | 80 | 0 | 0 | 50 | 5 | 95 | 0 | 95 | 5 | 100 | 75 | 25 | 20 | 100 | 85 | 10 | 0 | 5 | 100 | 100 | 10 | 100 | 95 | 100 | 100 | 45 | 0 | 0 | 5 | 10 | 5 | 90 | 100 | 100 | 65 | 0 | 0 | 85 | 0 | 90 | 0 | 100 |
| *C. braakii*[a] | 33 | 100 | 0 | 87 | 60 | 47 | 0 | 0 | 67 | 93 | 87 | 0 | 100 | 0 | 100 | 93 | 80 | 7 | 100 | 33 | 0 | 0 | 0 | 100 | 100 | 7 | 100 | 100 | 100 | 100 | 73 | 33 | 0 | 0 | 80 | 0 | 87 | 100 | 93 | 53 | 0 | 0 | 100 | 0 | 80 | 0 | 100 |
| *C. werkmanii*[a] | 0 | 100 | 0 | 100 | 100 | 100 | 0 | 0 | 100 | 0 | 100 | 0 | 100 | 100 | 100 | 100 | 17 | 0 | 100 | 0 | 0 | 0 | 0 | 100 | 100 | 0 | 100 | 100 | 100 | 100 | 0 | 0 | 0 | 0 | 0 | 0 | 100 | 100 | 100 | 100 | 0 | 0 | 100 | 0 | 100 | 0 | 100 |
| *C. sedlakii*[a] | 83 | 100 | 0 | 83 | 0 | 100 | 0 | 0 | 100 | 100 | 100 | 0 | 100 | 100 | 100 | 100 | 100 | 0 | 100 | 100 | 17 | 0 | 0 | 100 | 100 | 0 | 100 | 100 | 100 | 100 | 100 | 0 | 0 | 17 | 100 | 0 | 83 | 100 | 100 | 83 | 0 | 0 | 100 | 0 | 100 | 0 | 100 |
| *C. rodentium*[a] | 0 | 100 | 0 | 0 | 0 | 100 | 0 | 0 | 0 | 100 | 0 | 0 | 0 | 100 | 100 | 100 | 100 | 0 | 100 | 0 | 0 | 0 | 0 | 100 | 100 | 0 | 100 | 100 | 100 | 100 | 100 | 0 | 0 | 0 | 0 | 0 | 0 | 100 | 100 | 0 | 0 | 0 | 100 | 0 | 100 | 0 | 100 |
| *Citrobacter species 10*[a] | 0 | 100 | 0 | 33 | 67 | 0 | 0 | 0 | 33 | 0 | 67 | 0 | 100 | 100 | 100 | 100 | 67 | 33 | 100 | 0 | 0 | 0 | 0 | 100 | 100 | 0 | 100 | 100 | 100 | 100 | 67 | 0 | 0 | 0 | 67 | 0 | 67 | 67 | 100 | 0 | 0 | 0 | 100 | 0 | 67 | 0 | 100 |
| *Citrobacter species 11*[a] | 100 | 100 | 0 | 100 | 67 | 67 | 0 | 0 | 67 | 0 | 100 | 0 | 100 | 0 | 100 | 100 | 67 | 33 | 100 | 100 | 33 | 0 | 0 | 100 | 100 | 33 | 100 | 100 | 100 | 100 | 100 | 0 | 0 | 0 | 33 | 0 | 100 | 100 | 100 | 33 | 0 | 0 | 100 | 0 | 100 | 0 | 100 |
| ***Edwardsiella*** | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| *E. tarda*[a] | 99 | 100 | 0 | 1 | 100 | 0 | 0 | 100 | 0 | 100 | 98 | 0 | 0 | 0 | 100 | 100 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 9 | 0 | 0 | 100 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 30 | 0 | 25 | 0 | 0 | 0 | 100 | 0 | 0 | 0 | 100 |
| *E. tarda* biogroup 1[a] | 100 | 100 | 0 | 0 | 0 | 0 | 0 | 100 | 0 | 100 | 100 | 0 | 0 | 0 | 100 | 50 | 0 | 100 | 100 | 0 | 0 | 0 | 0 | 0 | 100 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 100 | 0 | 0 | 0 | 100 |
| *E. hoshinae*[a] | 50 | 100 | 0 | 0 | 0 | 0 | 0 | 100 | 0 | 95 | 100 | 0 | 0 | 100 | 100 | 35 | 0 | 100 | 100 | 0 | 50 | 0 | 0 | 0 | 13 | 0 | 0 | 100 | 0 | 100 | 0 | 0 | 0 | 0 | 0 | 0 | 65 | 0 | 0 | 0 | 0 | 0 | 100 | 0 | 0 | 0 | 100 |
| *E. ictaluri* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 100 | 0 | 65 | 0 | 0 | 0 | 0 | 100 | 50 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 100 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 100 | 0 | 0 | 0 | 100 |
| ***Enterobacter*** | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| *E. aerogenes*[a] | 0 | 5 | 98 | 95 | 0 | 2 | 0 | 98 | 0 | 98 | 97 | 0 | 98 | 95 | 100 | 100 | 95 | 100 | 100 | 5 | 100 | 98 | 95 | 100 | 100 | 96 | 99 | 99 | 100 | 100 | 100 | 95 | 0 | 98 | 99 | 100 | 98 | 90 | 95 | 50 | 0 | 0 | 100 | 0 | 100 | 0 | 95 |
| *E. cloacae*[a] | 0 | 5 | 100 | 100 | 0 | 65 | 0 | 0 | 97 | 96 | 95 | 0 | 98 | 75 | 100 | 100 | 93 | 97 | 100 | 15 | 75 | 25 | 15 | 95 | 100 | 97 | 92 | 100 | 99 | 100 | 99 | 85 | 0 | 30 | 90 | 15 | 40 | 75 | 30 | 75 | 0 | 0 | 99 | 0 | 99 | 0 | 100 |
| *E. agglomerans* group[a] | 20 | 50 | 70 | 50 | 0 | 20 | 20 | 0 | 0 | 0 | 85 | 2 | 35 | 65 | 100 | 20 | 40 | 75 | 100 | 15 | 65 | 7 | 15 | 30 | 95 | 30 | 85 | 89 | 93 | 97 | 55 | 7 | 0 | 60 | 50 | 50 | 30 | 40 | 25 | 30 | 0 | 0 | 85 | 0 | 90 | 75 | 98 |
| *E. gergoviae*[a] | 0 | 5 | 100 | 99 | 0 | 93 | 0 | 90 | 0 | 100 | 90 | 0 | 0 | 96 | 100 | 98 | 55 | 98 | 99 | 0 | 99 | 0 | 0 | 0 | 99 | 97 | 99 | 100 | 99 | 100 | 99 | 2 | 0 | 97 | 97 | 97 | 100 | 2 | 97 | 93 | 0 | 0 | 99 | 0 | 97 | 0 | 100 |
| *E. sakazakii*[a] | 11 | 5 | 100 | 99 | 0 | 1 | 50 | 0 | 99 | 91 | 96 | 0 | 99 | 18 | 100 | 98 | 99 | 100 | 100 | 5 | 99 | 0 | 75 | 0 | 100 | 99 | 100 | 100 | 100 | 100 | 100 | 96 | 0 | 100 | 100 | 0 | 15 | 1 | 1 | 96 | 0 | 0 | 99 | 0 | 100 | 98 | 100 |
| *E. taylorae*[a] *(cancerogenus)* | 0 | 5 | 100 | 100 | 0 | 1 | 0 | 0 | 94 | 99 | 99 | 0 | 98 | 100 | 100 | 100 | 10 | 0 | 100 | 0 | 92 | 0 | 0 | 1 | 100 | 0 | 100 | 99 | 100 | 100 | 100 | 1 | 0 | 90 | 0 | 0 | 1 | 75 | 0 | 35 | 0 | 0 | 100 | 0 | 100 | 0 | 100 |
| *E. amnigenus* biogroup 1[a] | 0 | 7 | 100 | 70 | 0 | 0 | 0 | 0 | 9 | 55 | 92 | 0 | 100 | 91 | 100 | 100 | 70 | 100 | 100 | 0 | 91 | 0 | 0 | 9 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 55 | 0 | 91 | 100 | 0 | 0 | 35 | 9 | 0 | 0 | 0 | 100 | 0 | 91 | 0 | 100 |
| *E. amnigenus* biogroup 2[a] | 0 | 65 | 100 | 100 | 0 | 0 | 0 | 0 | 35 | 100 | 100 | 0 | 100 | 100 | 100 | 100 | 35 | 0 | 100 | 0 | 100 | 0 | 0 | 100 | 100 | 0 | 100 | 100 | 100 | 100 | 100 | 100 | 0 | 100 | 100 | 0 | 0 | 100 | 0 | 0 | 0 | 0 | 100 | 0 | 100 | 0 | 100 |
| *E. asburiae*[a] | 0 | 100 | 2 | 100 | 0 | 60 | 0 | 0 | 21 | 95 | 0 | 0 | 97 | 3 | 100 | 95 | 75 | 100 | 100 | 0 | 100 | 0 | 0 | 100 | 100 | 70 | 5 | 100 | 97 | 100 | 100 | 95 | 0 | 95 | 0 | 0 | 11 | 21 | 30 | 87 | 0 | 0 | 100 | 0 | 100 | 0 | 100 |

(continued on next page)

*(Continued)*

TABLE 1 Biochemical reactions of the named species, biogroups, and Enteric Groups of the family *Enterobacteriaceae*[a] (*Continued*)

| Organism | Indole production | Methyl red | Voges-Proskauer | Citrate (Simmons) | Hydrogen sulfide (TSI) | Urea hydrolysis | Phenylalanine deaminase | Lysine decarboxylase | Arginine dihydrolase | Ornithine decarboxylase | Motility (36 °C) | Gelatin hydrolysis (22 °C) | Growth in KCN | Malonate utilization | D-Glucose, acid | D-Glucose, gas | Lactose fermentation | Sucrose fermentation | D-Mannitol fermentation | Dulcitol fermentation | Salicin fermentation | Adonitol fermentation | *myo*-Inositol fermentation | D-Sorbitol fermentation | L-Arabinose fermentation | Raffinose fermentation | L-Rhamnose fermentation | Maltose fermentation | D-Xylose fermentation | Trehalose fermentation | Cellobiose fermentation | alpha-Methyl-D-glucoside fermentation | Erythritol fermentation | Esculin hydrolysis | Melibiose fermentation | D-Arabitol fermentation | Glycerol fermentation | Mucate fermentation | Tartrate, Jordan's | Acetate utilization | Lipase (corn oil) | DNase at 25 °C | Nitrate → Nitrite | Coldase, Kovacs | ONPG test | Yellow pigment | D-Mannose fermentation |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Enterobacter* (continued) | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| *E. hormaechei*[a] | 0 | 57 | 100 | 96 | 0 | 87 | 4 | 0 | 78 | 91 | 52 | 0 | 100 | 100 | 100 | 83 | 9 | 100 | 100 | 87 | 44 | 0 | 0 | 0 | 100 | 0 | 100 | 100 | 98 | 100 | 100 | 83 | 0 | 0 | 0 | 0 | 4 | 96 | 13 | 74 | 0 | 0 | 100 | 0 | 95 | 0 | 100 |
| *E. intermedium*[a] | 0 | 100 | 100 | 65 | 0 | 0 | 0 | 0 | 0 | 89 | 89 | 0 | 65 | 100 | 100 | 100 | 100 | 65 | 100 | 100 | 100 | 0 | 0 | 0 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 0 | 100 | 100 | 0 | 100 | 100 | 100 | 0 | 0 | 0 | 100 | 0 | 100 | 0 | 100 |
| *E. cancerogenus* | 0 | 0 | 100 | 100 | 0 | 0 | 0 | 0 | 100 | 100 | 100 | 0 | 100 | 100 | 100 | 100 | 0 | 0 | 0 | 0 | 100 | 0 | 0 | 0 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 0 | 0 | 100 | 0 | 0 | 100 | 0 | 33 | 0 | 0 | 100 | 0 | 100 | 0 | 100 |
| *E. dissolvens* | 0 | 0 | 100 | 100 | 0 | 100 | 0 | 0 | 100 | 100 | 100 | 0 | 100 | 100 | 100 | 100 | 0 | 0 | 100 | 0 | 100 | 0 | 0 | 0 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 0 | 0 | 100 | 0 | 0 | 100 | 0 | 0 | 0 | 0 | 100 | 0 | 100 | 0 | 100 |
| *E. nimipressuralis* | 0 | 100 | 100 | 0 | 0 | 0 | 0 | 0 | 0 | 100 | 0 | 0 | 100 | 100 | 100 | 100 | 0 | 100 | 100 | 0 | 100 | 0 | 0 | 0 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 0 | 100 | 0 | 0 | 100 | 0 | 0 | 0 | 0 | 100 | 0 | 100 | 0 | 100 |
| *E. pyrinus* | 0 | 29 | 86 | 0 | 0 | 86 | 0 | 100 | 0 | 100 | 43 | 0 | 0 | 86 | 100 | 100 | 14 | 100 | 100 | 0 | 100 | 0 | 100 | 0 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 0 | 0 | 100 | 14 | 100 | 14 | 0 | 86 | 14 | 0 | 0 | 100 | 0 | 100 | 0 | 100 |
| *Escherichia* | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| *E. coli*[a] | 98 | 99 | 0 | 1 | 1 | 1 | 0 | 90 | 17 | 65 | 95 | 0 | 3 | 0 | 100 | 95 | 95 | 50 | 98 | 60 | 40 | 5 | 1 | 94 | 99 | 50 | 80 | 95 | 95 | 98 | 2 | 0 | 0 | 35 | 75 | 5 | 75 | 95 | 95 | 90 | 0 | 0 | 100 | 0 | 95 | 0 | 98 |
| *E. coli*, inactive[a] | 80 | 95 | 0 | 1 | 1 | 1 | 0 | 40 | 3 | 20 | 5 | 0 | 1 | 0 | 100 | 5 | 25 | 15 | 93 | 40 | 10 | 3 | 1 | 75 | 85 | 15 | 65 | 80 | 70 | 90 | 2 | 0 | 0 | 5 | 40 | 5 | 65 | 30 | 85 | 40 | 0 | 0 | 98 | 0 | 45 | 0 | 97 |
| *E. fergusonii*[a] | 98 | 100 | 0 | 17 | 0 | 0 | 0 | 95 | 5 | 100 | 93 | 0 | 0 | 35 | 100 | 95 | 0 | 0 | 98 | 60 | 65 | 98 | 0 | 0 | 98 | 0 | 92 | 96 | 96 | 96 | 98 | 0 | 0 | 46 | 0 | 100 | 20 | 0 | 96 | 96 | 0 | 0 | 100 | 0 | 83 | 0 | 100 |
| *E. hermannii*[a] | 99 | 100 | 0 | 1 | 0 | 0 | 0 | 6 | 0 | 100 | 99 | 0 | 94 | 0 | 100 | 97 | 45 | 45 | 100 | 19 | 40 | 0 | 0 | 0 | 100 | 40 | 97 | 100 | 100 | 100 | 97 | 0 | 0 | 40 | 0 | 8 | 3 | 97 | 35 | 78 | 0 | 0 | 100 | 0 | 98 | 98 | 100 |
| *E. vulneris*[a] | 0 | 100 | 0 | 0 | 0 | 0 | 0 | 85 | 30 | 0 | 100 | 0 | 15 | 85 | 100 | 97 | 15 | 8 | 100 | 0 | 30 | 0 | 0 | 1 | 100 | 99 | 93 | 100 | 100 | 100 | 100 | 25 | 0 | 20 | 100 | 0 | 25 | 78 | 2 | 30 | 0 | 0 | 100 | 0 | 100 | 50 | 100 |
| *E. blattae* | 0 | 100 | 0 | 50 | 0 | 0 | 0 | 100 | 0 | 100 | 0 | 0 | 0 | 100 | 100 | 100 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 100 | 0 | 100 | 100 | 100 | 75 | 0 | 0 | 0 | 0 | 0 | 0 | 100 | 50 | 50 | 0 | 0 | 0 | 100 | 0 | 0 | 0 | 100 |
| *Shigella* | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| *S. dysenteriae* (Group A)[a] | 45 | 89 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 100 | 0 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 30 | 45 | 0 | 30 | 15 | 4 | 90 | 0 | 0 | 0 | 0 | 0 | 0 | 10 | 0 | 75 | 0 | 0 | 0 | 99 | 0 | 30 | 0 | 100 |
| *S. flexneri* (Group B)[a] | 50 | 100 | 0 | 0 | 0 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 0 | 0 | 100 | 3 | 1 | 1 | 95 | 1 | 0 | 0 | 0 | 29 | 60 | 40 | 5 | 30 | 2 | 65 | 0 | 0 | 0 | 0 | 55 | 1 | 10 | 0 | 30 | 8 | 0 | 0 | 99 | 0 | 1 | 0 | 100 |
| *S. boydii* (Group C)[a] | 25 | 100 | 0 | 0 | 0 | 0 | 0 | 0 | 18 | 2 | 0 | 0 | 0 | 0 | 100 | 0 | 1 | 0 | 97 | 5 | 0 | 0 | 0 | 43 | 94 | 0 | 1 | 20 | 11 | 85 | 0 | 0 | 0 | 0 | 15 | 0 | 50 | 0 | 50 | 0 | 0 | 0 | 100 | 0 | 10 | 0 | 100 |
| *S. sonnei* (Group D)[a] | 0 | 100 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 98 | 0 | 0 | 0 | 0 | 100 | 0 | 2 | 1 | 99 | 0 | 0 | 0 | 0 | 2 | 95 | 3 | 75 | 90 | 2 | 100 | 5 | 0 | 0 | 0 | 25 | 0 | 15 | 10 | 90 | 0 | 0 | 0 | 100 | 0 | 90 | 0 | 100 |
| *Ewingella* | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| *E. americana*[a] | 0 | 84 | 95 | 95 | 0 | 0 | 0 | 0 | 0 | 0 | 60 | 0 | 5 | 0 | 100 | 0 | 70 | 0 | 100 | 0 | 80 | 0 | 0 | 0 | 0 | 0 | 23 | 16 | 13 | 99 | 10 | 0 | 0 | 50 | 0 | 99 | 24 | 0 | 35 | 10 | 0 | 0 | 97 | 0 | 85 | 0 | 99 |
| *Hafnia* | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| *H. alvei*[a] | 0 | 40 | 85 | 10 | 0 | 4 | 0 | 100 | 6 | 98 | 85 | 0 | 85 | 50 | 100 | 98 | 5 | 10 | 99 | 0 | 13 | 0 | 0 | 0 | 95 | 2 | 97 | 100 | 98 | 95 | 15 | 0 | 0 | 7 | 0 | 0 | 95 | 0 | 70 | 15 | 0 | 0 | 100 | 0 | 90 | 0 | 100 |
| *H. alvei* biogroup 1 | 0 | 85 | 70 | 0 | 0 | 0 | 0 | 100 | 0 | 45 | 0 | 0 | 0 | 45 | 100 | 0 | 0 | 0 | 55 | 0 | 55 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 70 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 30 | 0 | 0 | 0 | 100 | 0 | 30 | 0 | 100 |
| *Klebsiella* | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| *K. pneumoniae*[a] | 0 | 10 | 98 | 98 | 0 | 95 | 0 | 98 | 0 | 0 | 0 | 0 | 98 | 93 | 100 | 97 | 98 | 99 | 99 | 30 | 99 | 90 | 85 | 99 | 99 | 99 | 99 | 98 | 99 | 99 | 98 | 90 | 0 | 99 | 99 | 98 | 97 | 90 | 95 | 75 | 0 | 0 | 99 | 0 | 99 | 0 | 99 |
| *K. oxytoca*[a] | 99 | 20 | 95 | 95 | 0 | 90 | 1 | 99 | 0 | 0 | 0 | 0 | 97 | 98 | 100 | 97 | 100 | 100 | 99 | 55 | 100 | 99 | 98 | 99 | 98 | 100 | 100 | 100 | 100 | 100 | 100 | 98 | 2 | 100 | 99 | 98 | 99 | 93 | 98 | 90 | 0 | 0 | 100 | 0 | 100 | 1 | 100 |
| *K. ornithinolytica*[a] | 100 | 96 | 70 | 100 | 0 | 100 | 0 | 100 | 0 | 100 | 0 | 0 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 10 | 100 | 100 | 95 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 0 | 100 | 100 | 100 | 100 | 98 | 100 | 95 | 0 | 0 | 100 | 0 | 100 | 0 | 100 |
| *K. planticola*[a] | 20 | 100 | 98 | 100 | 0 | 98 | 0 | 100 | 0 | 0 | 0 | 0 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 15 | 100 | 100 | 100 | 92 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 0 | 100 | 100 | 100 | 100 | 96 | 100 | 62 | 0 | 0 | 100 | 0 | 100 | 1 | 100 |
| *K. ozaenae*[a] | 0 | 98 | 0 | 30 | 0 | 10 | 0 | 40 | 6 | 3 | 0 | 0 | 88 | 3 | 100 | 50 | 30 | 20 | 100 | 2 | 97 | 97 | 55 | 65 | 88 | 90 | 55 | 95 | 95 | 98 | 92 | 70 | 0 | 60 | 97 | 95 | 65 | 25 | 50 | 2 | 0 | 0 | 80 | 0 | 80 | 0 | 100 |
| *K. rhinoscleromatis*[a] | 0 | 100 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 80 | 95 | 100 | 0 | 0 | 75 | 100 | 0 | 98 | 100 | 95 | 100 | 100 | 90 | 96 | 100 | 100 | 100 | 100 | 0 | 0 | 30 | 100 | 100 | 50 | 0 | 50 | 0 | 0 | 0 | 100 | 0 | 0 | 0 | 100 |
| *K. terrigena* | 0 | 60 | 100 | 40 | 0 | 0 | 0 | 100 | 0 | 20 | 0 | 0 | 100 | 100 | 100 | 80 | 100 | 100 | 100 | 20 | 100 | 100 | 80 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 0 | 100 | 100 | 100 | 100 | 100 | 100 | 20 | 0 | 0 | 100 | 0 | 100 | 0 | 100 |
| *Kluyvera* | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| *K. ascorbata*[a] | 92 | 100 | 0 | 96 | 0 | 0 | 0 | 97 | 0 | 100 | 98 | 0 | 92 | 96 | 100 | 93 | 98 | 98 | 100 | 25 | 100 | 0 | 0 | 40 | 100 | 98 | 100 | 100 | 99 | 100 | 100 | 98 | 0 | 99 | 99 | 0 | 40 | 90 | 35 | 50 | 0 | 0 | 100 | 0 | 100 | 0 | 100 |
| *K. cryocrescens*[a] | 90 | 100 | 0 | 80 | 0 | 0 | 0 | 23 | 0 | 100 | 90 | 0 | 86 | 86 | 100 | 95 | 95 | 81 | 95 | 0 | 100 | 0 | 0 | 45 | 100 | 100 | 100 | 100 | 91 | 100 | 100 | 95 | 0 | 100 | 100 | 0 | 5 | 81 | 19 | 88 | 0 | 0 | 100 | 0 | 100 | 0 | 100 |
| *K. georgiana*[a] | 100 | 100 | 0 | 100 | 0 | 0 | 0 | 100 | 0 | 100 | 100 | 0 | 83 | 50 | 100 | 17 | 83 | 100 | 100 | 33 | 100 | 0 | 0 | 0 | 100 | 100 | 83 | 100 | 100 | 100 | 100 | 100 | 0 | 100 | 100 | 0 | 33 | 83 | 50 | 83 | 0 | 0 | 100 | 0 | 100 | 0 | 100 |
| *K. cochleae* | 0 | 100 | 67 | 100 | 0 | 0 | 0 | 0 | 0 | 100 | 100 | 0 | 100 | 100 | 100 | 100 | 33 | 33 | 100 | 100 | 100 | 0 | 0 | 0 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 0 | 100 | 100 | 0 | 100 | 33 | 0 | 0 | 0 | 0 | 100 | 0 | 100 | 0 | 100 |
| *Leclercia* | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| *L. adecarboxylata*[a] | 100 | 100 | 0 | 0 | 0 | 48 | 0 | 0 | 0 | 0 | 79 | 0 | 97 | 93 | 100 | 97 | 93 | 66 | 100 | 86 | 100 | 93 | 0 | 0 | 100 | 66 | 100 | 100 | 100 | 100 | 100 | 0 | 0 | 100 | 100 | 96 | 3 | 93 | 83 | 28 | 0 | 0 | 100 | 0 | 100 | 37 | 100 |
| *Leminorella* | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| *L. grimontii*[a] | 0 | 100 | 0 | 100 | 100 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 100 | 33 | 0 | 0 | 0 | 83 | 0 | 0 | 0 | 0 | 100 | 0 | 0 | 0 | 83 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 17 | 100 | 100 | 0 | 0 | 0 | 100 | 0 | 0 | 0 | 0 |
| *L. richardii*[a] | 0 | 0 | 0 | 0 | 100 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 100 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 100 | 0 | 0 | 0 | 100 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 50 | 100 | 0 | 0 | 0 | 100 | 0 | 0 | 0 | 0 |

(*Continued*)

TABLE 1 (Continued)

| Organism | Indole production | Methyl red | Voges-Proskauer | Citrate (Simmons) | Hydrogen sulfide (TSI) | Urea hydrolysis | Phenylalanine deaminase | Lysine decarboxylase | Arginine dihydrolase | Ornithine decarboxylase | Motility (36 °C) | Gelatin hydrolysis (22 °C) | Growth in KCN | Malonate utilization | D-Glucose, acid | D-Glucose, gas | Lactose fermentation | Sucrose fermentation | D-Mannitol fermentation | Dulcitol fermentation | Salicin fermentation | Adonitol fermentation | myo-Inositol fermentation | D-Sorbitol fermentation | L-Arabinose fermentation | Raffinose fermentation | L-Rhamnose fermentation | Melibiose fermentation | D-Xylose fermentation | Trehalose fermentation | Cellobiose fermentation | alpha-Methyl-D-glucoside fermentation | Erythritol fermentation | Esculin hydrolysis | Melibiose fermentation | D-Arabitol fermentation | Glycerol fermentation | Mucate fermentation | Tartrate, Jordan's | Acetate utilization | Lipase (corn oil) | DNase at 25 °C | Nitrate → Nitrite | Oxidase, Kovacs | ONPG test | Yellow pigment | D-Mannose fermentation |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Moellerella* | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| *M. wisconsensis** | 0 | 100 | 0 | 80 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 70 | 0 | 100 | 0 | 100 | 100 | 60 | 0 | 0 | 100 | 0 | 0 | 0 | 100 | 0 | 30 | 0 | 0 | 0 | 0 | 0 | 0 | 100 | 75 | 10 | 0 | 30 | 10 | 0 | 0 | 90 | 0 | 90 | 0 | 100 |
| *Morganella* | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| *M. morganii ss morganii** | 95 | 95 | 0 | 0 | 20 | 95 | 95 | 1 | 0 | 95 | 95 | 0 | 98 | 1 | 99 | 90 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 5 | 0 | 95 | 0 | 0 | 0 | 90 | 0 | 10 | 0 | 0 |
| *M. morganii* biogroup 1* | 100 | 95 | 0 | 0 | 15 | 100 | 100 | 100 | 0 | 80 | 0 | 0 | 90 | 5 | 100 | 93 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 100 | 0 | 100 | 0 | 0 | 0 | 90 | 0 | 20 | 0 | 100 |
| *M. morganii ss sibonii* 1* | 50 | 86 | 0 | 0 | 7 | 100 | 93 | 29 | 0 | 64 | 79 | 0 | 79 | 0 | 100 | 86 | 0 | 7 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 100 | 0 | 0 | 0 | 0 | 0 | 0 | 7 | 7 | 100 | 0 | 0 | 0 | 100 | 0 | 0 | 0 | 100 |
| *Obesumbacterium* | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| *O. proteus* biogroup 2 | 0 | 15 | 0 | 0 | 0 | 0 | 0 | 100 | 0 | 100 | 0 | 0 | 0 | 0 | 100 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 15 | 50 | 15 | 85 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 15 | 0 | 0 | 0 | 100 | 0 | 0 | 0 | 85 |
| *Pragia* | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| *P. fontium* | 0 | 100 | 0 | 89 | 89 | 0 | 22 | 0 | 0 | 0 | 100 | 0 | 0 | 0 | 100 | 0 | 0 | 0 | 0 | 0 | 78 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 78 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 100 | 0 | 0 | 0 | 0 |
| *Pantoea* | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| *P. dispersa* | 0 | 82 | 64 | 100 | 0 | 0 | 9 | 0 | 0 | 0 | 100 | 0 | 82 | 9 | 100 | 0 | 0 | 1 | 100 | 0 | 0 | 0 | 0 | 0 | 100 | 0 | 91 | 82 | 100 | 100 | 55 | 0 | 0 | 0 | 0 | 100 | 27 | 0 | 9 | 100 | 0 | 0 | 91 | 0 | 91 | 27 | 100 |
| *Photorhabdus* | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| *P. luminescens* (25 °C) | 50 | 0 | 0 | 50 | 0 | 25 | 0 | 0 | 0 | 0 | 100 | 50 | 0 | 0 | 75 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 25 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 50 | 0 | 0 | 0 | 0 | 0 | 0 | 50 | 100 |
| *P.* DNA group 5* | 0 | 0 | 0 | 20 | 0 | 60 | 0 | 0 | 0 | 0 | 100 | 80 | 20 | 0 | 100 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 60 | 20 | 0 | 0 | 0 | 0 | 0 | 60 | 100 |
| *Proteus* | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| *P. mirabilis** | 2 | 97 | 50 | 65 | 98 | 98 | 98 | 0 | 0 | 99 | 95 | 90 | 98 | 2 | 100 | 96 | 2 | 15 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 98 | 98 | 1 | 0 | 0 | 0 | 0 | 0 | 70 | 0 | 87 | 20 | 92 | 50 | 95 | 0 | 0 | 0 | 0 |
| *P. vulgaris** | 98 | 95 | 0 | 15 | 95 | 95 | 99 | 0 | 0 | 0 | 95 | 91 | 99 | 0 | 100 | 85 | 2 | 97 | 0 | 0 | 50 | 0 | 0 | 0 | 0 | 0 | 1 | 5 | 97 | 95 | 30 | 0 | 60 | 1 | 50 | 0 | 0 | 60 | 0 | 80 | 25 | 80 | 80 | 98 | 0 | 1 | 0 | 0 |
| *P. penneri** | 0 | 100 | 0 | 0 | 30 | 100 | 99 | 0 | 0 | 0 | 85 | 50 | 99 | 0 | 100 | 45 | 1 | 100 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 100 | 100 | 55 | 0 | 80 | 0 | 0 | 0 | 0 | 55 | 0 | 85 | 5 | 45 | 40 | 90 | 0 | 1 | 0 | 0 |
| *P. myxofaciens* | 0 | 100 | 100 | 50 | 0 | 100 | 100 | 0 | 0 | 0 | 100 | 100 | 100 | 0 | 100 | 100 | 0 | 100 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 100 | 0 | 100 | 0 | 100 | 0 | 0 | 100 | 0 | 100 | 0 | 100 | 0 | 100 | 50 | 100 | 0 | 0 | 0 | 0 |
| *Providencia* | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| *P. rettgeri** | 99 | 93 | 0 | 95 | 0 | 98 | 98 | 0 | 0 | 0 | 94 | 0 | 97 | 0 | 100 | 10 | 5 | 15 | 100 | 0 | 50 | 100 | 90 | 1 | 0 | 5 | 70 | 2 | 10 | 0 | 3 | 2 | 75 | 35 | 5 | 100 | 60 | 0 | 95 | 60 | 0 | 0 | 100 | 0 | 5 | 0 | 100 |
| *P. stuartii** | 98 | 100 | 0 | 93 | 0 | 30 | 95 | 0 | 0 | 0 | 85 | 0 | 100 | 0 | 100 | 0 | 2 | 50 | 10 | 0 | 2 | 5 | 95 | 1 | 1 | 7 | 0 | 1 | 7 | 98 | 5 | 0 | 0 | 0 | 0 | 0 | 50 | 0 | 90 | 75 | 0 | 10 | 100 | 0 | 10 | 0 | 100 |
| *P. alcalifaciens** | 99 | 99 | 0 | 98 | 0 | 0 | 98 | 0 | 0 | 1 | 96 | 0 | 100 | 0 | 100 | 85 | 0 | 15 | 2 | 0 | 1 | 98 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 15 | 0 | 90 | 40 | 0 | 0 | 100 | 0 | 1 | 0 | 100 |
| *P. rustigianii** | 98 | 65 | 0 | 15 | 0 | 0 | 100 | 0 | 0 | 0 | 30 | 0 | 100 | 0 | 100 | 35 | 0 | 35 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 5 | 0 | 50 | 25 | 0 | 0 | 100 | 0 | 0 | 0 | 100 |
| *P. heimbachae* | 0 | 85 | 0 | 0 | 0 | 0 | 100 | 0 | 0 | 0 | 46 | 0 | 8 | 0 | 100 | 0 | 0 | 0 | 0 | 0 | 0 | 92 | 46 | 0 | 0 | 0 | 100 | 54 | 8 | 0 | 0 | 0 | 0 | 0 | 0 | 92 | 0 | 0 | 69 | 0 | 0 | 0 | 100 | 0 | 0 | 0 | 100 |
| *Rahnella* | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| *R.aquatilis** | 0 | 88 | 100 | 94 | 0 | 0 | 95 | 0 | 0 | 0 | 6 | 0 | 0 | 100 | 100 | 98 | 100 | 100 | 100 | 88 | 100 | 0 | 0 | 94 | 100 | 94 | 94 | 94 | 94 | 100 | 100 | 0 | 0 | 100 | 100 | 0 | 13 | 30 | 6 | 6 | 0 | 0 | 100 | 0 | 100 | 0 | 100 |
| *Salmonella* | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Group I[a] strains* | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Most serotypes* | 1 | 100 | 0 | 95 | 95 | 1 | 0 | 98 | 70 | 97 | 95 | 0 | 0 | 0 | 100 | 98 | 1 | 1 | 100 | 96 | 0 | 0 | 35 | 95 | 99 | 2 | 95 | 97 | 97 | 99 | 5 | 2 | 0 | 5 | 95 | 0 | 5 | 90 | 90 | 90 | 0 | 2 | 100 | 0 | 2 | 0 | 100 |
| Serotype Typhi* | 0 | 100 | 0 | 0 | 97 | 0 | 0 | 98 | 3 | 0 | 97 | 0 | 0 | 0 | 100 | 0 | 1 | 0 | 100 | 0 | 0 | 0 | 0 | 99 | 2 | 0 | 0 | 97 | 82 | 100 | 0 | 0 | 0 | 0 | 100 | 0 | 20 | 0 | 100 | 0 | 0 | 0 | 100 | 0 | 0 | 0 | 100 |
| Serotype Choleraesuis* | 0 | 100 | 0 | 25 | 50 | 0 | 0 | 95 | 55 | 100 | 95 | 0 | 0 | 0 | 100 | 95 | 0 | 0 | 98 | 5 | 0 | 0 | 0 | 90 | 0 | 1 | 100 | 95 | 98 | 0 | 0 | 0 | 1 | 0 | 45 | 1 | 0 | 0 | 85 | 1 | 0 | 0 | 98 | 0 | 0 | 0 | 95 |
| Serotype Paratyphi A* | 0 | 100 | 0 | 0 | 10 | 0 | 0 | 0 | 15 | 95 | 95 | 0 | 0 | 0 | 100 | 99 | 0 | 0 | 100 | 90 | 0 | 0 | 0 | 95 | 100 | 0 | 100 | 95 | 0 | 100 | 5 | 0 | 0 | 0 | 95 | 0 | 10 | 0 | 0 | 0 | 0 | 0 | 100 | 0 | 0 | 0 | 100 |
| Serotype Gallinarum* | 0 | 100 | 0 | 0 | 100 | 0 | 0 | 90 | 10 | 1 | 0 | 0 | 0 | 0 | 100 | 0 | 0 | 0 | 100 | 90 | 0 | 0 | 0 | 1 | 80 | 10 | 10 | 90 | 70 | 50 | 10 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 100 | 0 | 0 | 0 | 100 |
| Serotype Pullorum* | 0 | 90 | 0 | 0 | 90 | 0 | 0 | 100 | 10 | 95 | 0 | 0 | 0 | 0 | 100 | 90 | 0 | 0 | 100 | 0 | 0 | 0 | 0 | 10 | 100 | 1 | 100 | 5 | 90 | 90 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 100 | 0 | 0 | 0 | 100 |
| Group II strains* | 2 | 100 | 0 | 100 | 100 | 0 | 0 | 100 | 90 | 100 | 98 | 2 | 0 | 95 | 100 | 100 | 1 | 1 | 100 | 90 | 5 | 0 | 5 | 100 | 100 | 0 | 100 | 100 | 100 | 100 | 0 | 8 | 0 | 15 | 8 | 0 | 25 | 96 | 50 | 95 | 0 | 0 | 100 | 0 | 15 | 0 | 95 |
| Group IIIa strains* | 1 | 100 | 0 | 99 | 99 | 0 | 0 | 99 | 70 | 99 | 99 | 0 | 1 | 95 | 100 | 99 | 15 | 1 | 100 | 0 | 0 | 0 | 0 | 99 | 99 | 1 | 99 | 98 | 100 | 99 | 1 | 1 | 0 | 1 | 95 | 1 | 10 | 90 | 5 | 90 | 0 | 2 | 100 | 0 | 100 | 0 | 100 |
| Group IIIb strains* | 2 | 100 | 0 | 98 | 99 | 0 | 0 | 99 | 70 | 99 | 99 | 0 | 1 | 95 | 100 | 99 | 85 | 5 | 100 | 1 | 0 | 0 | 0 | 89 | 99 | 1 | 99 | 98 | 100 | 99 | 1 | 1 | 0 | 1 | 95 | 1 | 10 | 30 | 20 | 75 | 0 | 2 | 100 | 0 | 92 | 0 | 100 |
| Group IV strains* | 0 | 100 | 0 | 98 | 100 | 2 | 0 | 100 | 70 | 100 | 98 | 0 | 95 | 0 | 100 | 100 | 0 | 0 | 98 | 0 | 60 | 5 | 0 | 100 | 100 | 0 | 98 | 100 | 100 | 100 | 50 | 0 | 0 | 0 | 100 | 5 | 0 | 0 | 65 | 70 | 0 | 0 | 100 | 0 | 0 | 0 | 100 |
| Group V strains* | 0 | 100 | 0 | 94 | 100 | 0 | 0 | 100 | 94 | 100 | 100 | 0 | 100 | 0 | 100 | 94 | 0 | 0 | 100 | 94 | 0 | 0 | 0 | 100 | 94 | 0 | 88 | 100 | 100 | 100 | 0 | 0 | 0 | 0 | 94 | 0 | 0 | 88 | 0 | 100 | 0 | 0 | 100 | 0 | 94 | 0 | 100 |
| Group VI strains* | 0 | 100 | 0 | 89 | 100 | 0 | 0 | 100 | 67 | 100 | 100 | 0 | 0 | 0 | 100 | 100 | 22 | 0 | 100 | 67 | 0 | 0 | 0 | 0 | 100 | 0 | 100 | 100 | 100 | 100 | 0 | 0 | 0 | 0 | 89 | 0 | 33 | 89 | 100 | 89 | 0 | 0 | 100 | 0 | 44 | 0 | 100 |

*(Continued)*

TABLE 1 Biochemical reactions of the named species, biogroups, and Enteric Groups of the family *Enterobacteriaceae*[a] (*Continued*)

| Organism | Indole production | Methyl red | Voges-Proskauer | Citrate (Simmons) | Hydrogen sulfide (TSI) | Urea hydrolysis | Phenylalanine deaminase | Lysine decarboxylase | Arginine dihydrolase | Ornithine decarboxylase | Motility (36°C) | Gelatin hydrolysis (22°C) | Growth in KCN | Malonate utilization | D-Glucose, acid | D-Glucose, gas | Lactose fermentation | Sucrose fermentation | D-Mannitol fermentation | Dulcitol fermentation | Salicin fermentation | Adonitol fermentation | *myo*-Inositol fermentation | D-Sorbitol fermentation | L-Arabinose fermentation | Raffinose fermentation | L-Rhamnose fermentation | Maltose fermentation | D-Xylose fermentation | Trehalose fermentation | Cellobiose fermentation | alpha-Methyl-D-glucoside fermentation | Erythritol fermentation | Esculin hydrolysis | Melibiose fermentation | D-Arabitol fermentation | Glycerol fermentation | Mucate fermentation | Tartrate, Jordan's | Acetate utilization | Lipase (corn oil) | DNase at 25°C | Nitrate → Nitrite | Oxidase, Kovacs | ONPG test | Yellow pigment | D-Mannose fermentation |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Serratia* | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| *S. marcescens*[b] | 1 | 20 | 98 | 98 | 0 | 15 | 0 | 99 | 0 | 99 | 97 | 90 | 95 | 3 | 100 | 55 | 2 | 99 | 99 | 0 | 95 | 40 | 75 | 99 | 0 | 2 | 0 | 96 | 7 | 99 | 5 | 0 | 1 | 95 | 0 | 0 | 95 | 0 | 75 | 50 | 98 | 98 | 98 | 0 | 95 | 0 | 99 |
| *S. marcescens* biogroup 1[b] | 0 | 100 | 60 | 30 | 0 | 0 | 0 | 55 | 4 | 65 | 17 | 30 | 70 | 0 | 100 | 0 | 4 | 100 | 98 | 0 | 92 | 30 | 30 | 92 | 0 | 0 | 0 | 70 | 0 | 100 | 4 | 0 | 0 | 96 | 0 | 0 | 92 | 0 | 50 | 4 | 75 | 82 | 83 | 0 | 75 | 0 | 100 |
| *S. liquefaciens* group[b] | 1 | 93 | 93 | 90 | 0 | 3 | 0 | 95 | 0 | 95 | 95 | 90 | 90 | 2 | 100 | 75 | 10 | 98 | 100 | 0 | 97 | 5 | 60 | 95 | 98 | 85 | 15 | 98 | 100 | 100 | 5 | 5 | 0 | 97 | 75 | 0 | 95 | 0 | 75 | 40 | 85 | 85 | 100 | 0 | 93 | 0 | 100 |
| *S. rubidaea*[b] | 0 | 20 | 100 | 95 | 0 | 2 | 0 | 55 | 0 | 0 | 85 | 90 | 25 | 94 | 100 | 30 | 100 | 99 | 100 | 0 | 99 | 99 | 20 | 1 | 100 | 99 | 1 | 99 | 99 | 100 | 94 | 1 | 0 | 94 | 99 | 85 | 20 | 0 | 70 | 80 | 99 | 99 | 100 | 0 | 100 | 0 | 100 |
| *S. odorifera* biogroup 1[b] | 60 | 100 | 50 | 100 | 0 | 5 | 0 | 100 | 0 | 100 | 100 | 95 | 60 | 0 | 100 | 0 | 70 | 100 | 100 | 0 | 98 | 50 | 100 | 100 | 100 | 100 | 95 | 100 | 100 | 100 | 100 | 0 | 0 | 95 | 100 | 0 | 40 | 5 | 100 | 60 | 35 | 100 | 100 | 0 | 100 | 0 | 100 |
| *S. odorifera* biogroup 2[b] | 50 | 60 | 100 | 97 | 0 | 0 | 0 | 94 | 0 | 0 | 100 | 94 | 19 | 0 | 100 | 13 | 97 | 0 | 97 | 0 | 45 | 55 | 100 | 100 | 100 | 7 | 94 | 100 | 100 | 100 | 100 | 0 | 7 | 40 | 96 | 0 | 50 | 0 | 100 | 65 | 65 | 100 | 100 | 0 | 100 | 0 | 100 |
| *S. plymuthica*[b] | 0 | 94 | 80 | 75 | 0 | 0 | 0 | 0 | 0 | 0 | 50 | 60 | 30 | 0 | 100 | 40 | 80 | 100 | 100 | 0 | 94 | 0 | 50 | 65 | 100 | 94 | 0 | 94 | 94 | 100 | 88 | 70 | 0 | 81 | 93 | 0 | 50 | 0 | 100 | 55 | 70 | 100 | 100 | 0 | 70 | 0 | 100 |
| *S. ficaria*[b] | 0 | 75 | 75 | 100 | 0 | 0 | 0 | 0 | 0 | 0 | 100 | 100 | 55 | 0 | 100 | 0 | 15 | 100 | 100 | 0 | 100 | 0 | 55 | 100 | 100 | 70 | 35 | 100 | 100 | 100 | 100 | 8 | 0 | 100 | 40 | 100 | 0 | 0 | 17 | 40 | 77 | 100 | 92 | 8 | 100 | 0 | 100 |
| *S. entomophila* | 0 | 20 | 100 | 100 | 0 | 0 | 0 | 0 | 0 | 0 | 100 | 100 | 100 | 0 | 100 | 0 | 0 | 100 | 100 | 0 | 100 | 0 | 0 | 0 | 0 | 0 | 0 | 100 | 40 | 100 | 0 | 0 | 0 | 100 | 0 | 60 | 0 | 0 | 100 | 80 | 20 | 100 | 100 | 0 | 100 | 0 | 100 |
| *"Serratia" fonticola*[b] | 0 | 100 | 9 | 91 | 0 | 13 | 0 | 100 | 0 | 97 | 91 | 0 | 70 | 88 | 100 | 79 | 97 | 21 | 100 | 91 | 100 | 100 | 30 | 100 | 100 | 100 | 76 | 97 | 85 | 100 | 6 | 91 | 0 | 100 | 98 | 100 | 88 | 0 | 58 | 15 | 0 | 0 | 100 | 0 | 100 | 0 | 100 |
| *Tatumella* | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| *T. ptyseos*[b] | 0 | 0 | 5 | 2 | 0 | 0 | 90 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 100 | 0 | 0 | 98 | 0 | 0 | 55 | 0 | 0 | 0 | 0 | 11 | 0 | 0 | 9 | 93 | 0 | 0 | 0 | 0 | 25 | 0 | 7 | 0 | 0 | 0 | 0 | 98 | 0 | 0 | 0 | 100 |
| *Trabulsiella* | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| *T. guamensis*[b] | 40 | 100 | 0 | 88 | 100 | 0 | 0 | 100 | 50 | 100 | 100 | 0 | 100 | 0 | 100 | 100 | 0 | 0 | 100 | 0 | 13 | 0 | 0 | 100 | 100 | 0 | 100 | 100 | 100 | 100 | 100 | 0 | 0 | 40 | 0 | 0 | 0 | 100 | 50 | 88 | 0 | 0 | 100 | 0 | 100 | 0 | 100 |
| *Xenorhabdus* | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| *X. nematophilus* (25°C) | 40 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 100 | 80 | 0 | 0 | 80 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 60 | 0 | 0 | 20 | 20 | 0 | 0 | 60 | 80 |
| *Yersinia* | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| *Y. enterocolitica*[b] | 50 | 97 | 2 | 0 | 0 | 75 | 0 | 0 | 0 | 95 | 2 | 0 | 2 | 0 | 100 | 5 | 5 | 95 | 98 | 0 | 20 | 0 | 30 | 99 | 98 | 5 | 1 | 75 | 70 | 98 | 75 | 0 | 0 | 25 | 1 | 40 | 90 | 0 | 85 | 15 | 55 | 5 | 98 | 0 | 95 | 0 | 100 |
| *Y. frederiksenii*[b] | 100 | 100 | 0 | 15 | 0 | 70 | 0 | 0 | 0 | 95 | 5 | 0 | 0 | 0 | 100 | 40 | 40 | 100 | 100 | 0 | 92 | 0 | 20 | 100 | 100 | 30 | 99 | 100 | 100 | 100 | 100 | 0 | 0 | 85 | 0 | 100 | 85 | 5 | 55 | 15 | 55 | 0 | 100 | 0 | 100 | 0 | 100 |
| *Y. intermedia*[b] | 100 | 100 | 5 | 5 | 0 | 80 | 0 | 0 | 0 | 100 | 5 | 0 | 10 | 5 | 100 | 18 | 35 | 100 | 100 | 0 | 100 | 0 | 15 | 100 | 100 | 45 | 100 | 100 | 100 | 100 | 96 | 77 | 0 | 100 | 80 | 45 | 60 | 6 | 88 | 18 | 12 | 0 | 94 | 0 | 90 | 0 | 100 |
| *Y. kristensenii*[b] | 30 | 92 | 0 | 0 | 0 | 77 | 0 | 0 | 0 | 92 | 5 | 0 | 0 | 0 | 100 | 23 | 8 | 0 | 100 | 0 | 15 | 0 | 15 | 100 | 77 | 0 | 0 | 100 | 85 | 100 | 100 | 0 | 0 | 0 | 0 | 45 | 70 | 0 | 40 | 8 | 0 | 0 | 100 | 0 | 70 | 0 | 100 |
| *Y. rohdei*[b] | 0 | 62 | 0 | 0 | 0 | 62 | 0 | 0 | 0 | 25 | 0 | 0 | 0 | 0 | 100 | 0 | 0 | 100 | 100 | 0 | 0 | 0 | 0 | 0 | 100 | 62 | 0 | 0 | 38 | 100 | 25 | 0 | 0 | 0 | 50 | 0 | 38 | 0 | 100 | 0 | 0 | 0 | 88 | 0 | 50 | 0 | 100 |
| *Y. aldovae* | 0 | 80 | 0 | 0 | 0 | 60 | 0 | 0 | 0 | 40 | 0 | 0 | 0 | 0 | 100 | 0 | 0 | 20 | 80 | 0 | 0 | 0 | 0 | 60 | 60 | 0 | 0 | 0 | 40 | 80 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 100 | 0 | 0 | 0 | 100 | 0 | 0 | 0 | 100 |
| *Y. bercovieri*[b] | 0 | 100 | 0 | 0 | 0 | 60 | 0 | 0 | 0 | 80 | 0 | 0 | 0 | 0 | 100 | 0 | 20 | 100 | 100 | 0 | 20 | 0 | 0 | 100 | 100 | 0 | 0 | 100 | 100 | 100 | 100 | 0 | 0 | 20 | 0 | 0 | 0 | 0 | 100 | 0 | 0 | 0 | 100 | 0 | 80 | 0 | 100 |
| *Y. mollaretii*[b] | 0 | 100 | 0 | 0 | 0 | 20 | 0 | 0 | 0 | 80 | 0 | 0 | 0 | 0 | 100 | 0 | 40 | 100 | 100 | 0 | 20 | 0 | 0 | 100 | 100 | 0 | 0 | 60 | 60 | 100 | 100 | 0 | 0 | 0 | 0 | 0 | 20 | 0 | 100 | 0 | 0 | 0 | 100 | 0 | 20 | 0 | 100 |
| *Y. pestis*[b] | 0 | 80 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 100 | 0 | 0 | 0 | 97 | 0 | 70 | 0 | 0 | 50 | 100 | 0 | 1 | 80 | 90 | 100 | 0 | 0 | 0 | 50 | 20 | 0 | 50 | 0 | 0 | 0 | 0 | 0 | 85 | 0 | 50 | 0 | 100 |
| *Y. pseudotuberculosis*[b] | 0 | 100 | 0 | 0 | 0 | 95 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 100 | 0 | 0 | 0 | 100 | 0 | 25 | 0 | 0 | 0 | 50 | 15 | 70 | 95 | 100 | 100 | 0 | 0 | 0 | 95 | 70 | 0 | 50 | 0 | 50 | 0 | 0 | 0 | 95 | 0 | 70 | 0 | 100 |
| *"Yersinia" ruckeri*[b] | 0 | 97 | 10 | 0 | 0 | 0 | 0 | 50 | 5 | 100 | 0 | 30 | 15 | 0 | 100 | 5 | 0 | 0 | 100 | 0 | 0 | 0 | 0 | 50 | 5 | 5 | 0 | 95 | 0 | 95 | 5 | 0 | 0 | 0 | 0 | 0 | 30 | 0 | 30 | 0 | 30 | 0 | 75 | 0 | 50 | 0 | 100 |
| *Yokenella (Koserella)* | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| *Y. regensburgei*[b] | 0 | 100 | 0 | 92 | 0 | 0 | 0 | 100 | 8 | 100 | 100 | 0 | 92 | 0 | 100 | 100 | 0 | 0 | 100 | 0 | 8 | 0 | 0 | 0 | 100 | 25 | 100 | 100 | 100 | 100 | 100 | 0 | 0 | 67 | 92 | 0 | 0 | 0 | 0 | 25 | 0 | 0 | 100 | 0 | 100 | 0 | 100 |
| Enteric Group 58[b] | 0 | 100 | 0 | 85 | 0 | 70 | 0 | 100 | 0 | 85 | 100 | 0 | 100 | 85 | 100 | 85 | 30 | 0 | 100 | 85 | 100 | 0 | 0 | 100 | 100 | 0 | 100 | 100 | 100 | 100 | 100 | 55 | 0 | 0 | 0 | 0 | 30 | 0 | 60 | 45 | 0 | 0 | 100 | 0 | 100 | 0 | 100 |
| Enteric Group 59[b] | 10 | 100 | 0 | 100 | 0 | 0 | 30 | 0 | 60 | 0 | 100 | 0 | 80 | 90 | 100 | 100 | 80 | 0 | 100 | 0 | 100 | 0 | 0 | 0 | 100 | 0 | 100 | 100 | 100 | 100 | 100 | 10 | 0 | 100 | 0 | 10 | 10 | 60 | 50 | 50 | 0 | 0 | 100 | 0 | 100 | 25 | 100 |
| Enteric Group 60[b] | 0 | 100 | 0 | 0 | 0 | 50 | 0 | 0 | 0 | 100 | 75 | 0 | 0 | 100 | 100 | 100 | 0 | 0 | 50 | 0 | 0 | 0 | 0 | 0 | 25 | 0 | 75 | 0 | 0 | 100 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 75 | 0 | 75 | 0 | 0 | 100 | 0 | 100 | 0 | 100 |
| Enteric Group 63 | 0 | 100 | 0 | 0 | 0 | 0 | 0 | 100 | 0 | 100 | 65 | 0 | 0 | 0 | 100 | 100 | 0 | 0 | 100 | 0 | 100 | 0 | 0 | 100 | 100 | 0 | 100 | 100 | 100 | 100 | 100 | 65 | 0 | 100 | 0 | 0 | 0 | 65 | 0 | 0 | 0 | 0 | 100 | 0 | 100 | 0 | 100 |
| Enteric Group 64 | 0 | 100 | 0 | 50 | 0 | 0 | 0 | 0 | 50 | 0 | 100 | 0 | 100 | 100 | 100 | 50 | 100 | 0 | 100 | 0 | 100 | 100 | 0 | 0 | 100 | 0 | 100 | 100 | 100 | 100 | 100 | 0 | 0 | 100 | 0 | 100 | 0 | 100 | 50 | 0 | 0 | 0 | 100 | 0 | 100 | 0 | 100 |
| Enteric Group 68[b] | 0 | 100 | 50 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 100 | 0 | 100 | 0 | 0 | 100 | 100 | 0 | 50 | 0 | 0 | 0 | 0 | 0 | 0 | 50 | 0 | 100 | 0 | 0 | 0 | 0 | 0 | 0 | 50 | 0 | 0 | 0 | 0 | 100 | 100 | 0 | 0 | 0 | 100 |
| Enteric Group 69 | 0 | 0 | 100 | 100 | 0 | 0 | 0 | 0 | 100 | 100 | 100 | 0 | 100 | 100 | 100 | 100 | 100 | 25 | 100 | 100 | 100 | 0 | 0 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 0 | 100 | 100 | 0 | 0 | 100 | 0 | 25 | 0 | 0 | 100 | 0 | 100 | 0 | 100 |

[a] Each number is the percentage of positive reactions after 2 days of incubation at 36°C (unless a different temperature is indicated). The vast majority of these positive reactions occur within 24 h. Reactions that become positive after 2 days are not considered. Abbreviations: TSI, triple sugar iron agar; ONPG, *o*-nitrophenyl-β-D-galactopyranoside.

[b] An "*" indicates that the organism occurs in human clinical specimens.

[c] The roman numerals refer to the seven *Salmonella* subgroups that are biochemically and genetically distinct.

TABLE 2 Distribution of *Enterobacteriaceae*, *Vibrionaceae*, and other organisms in patients with bacteremia[a]

| Organism | No. of cases/yr[b] in England and Wales |
|---|---|
| Family *Enterobacteriaceae* | |
| *Escherichia coli* | 9,680 |
| *Klebsiella* species | 2,075 |
| *Proteus* species | 1,516 |
| *Enterobacter* species | 1,316 |
| *Salmonella*, all serotypes | 413 |
| *Salmonella*, serotypes Typhi and Paratyphi | 140 |
| *Serratia* species | 368 |
| *Citrobacter* species | 326 |
| *Providencia* species | 55 |
| Family *Vibrionaceae* | |
| *Aeromonas* species | 40 |
| Other organisms (for comparison) | |
| *Staphyloccus aureus* | 9,016 |
| *Streptococcus pneumoniae* | 5,871 |
| *Enterococcus* species | 2,821 |
| *Pseudomonas aeruginosa* | 1,154 |
| *Neisseria meningitidis* | 1,096 |
| *Bacteroides* species | 907 |
| *Streptococcus* species, group B | 766 |
| *Pseudomonas* species | 765 |
| *Acinetobacter* species | 557 |
| *Clostridium* species | 380 |
| *Hemophilus influenzae* | 348 |
| *Bacillus* species | 255 |
| *Branhamella/Moraxella* species | 70 |
| *Listeria* species | 67 |
| *Campylobacter* species | 57 |
| *Mycobacterium avium-intracellulare* | 40 |

[a] Data are for bacteremia (blood, bone marrow, spleen, or heart) from published surveillance reports for England and Wales. Bacteremia is not a reportable disease in the United States.

[b] Data are adapted from the *CDR Weekly, Communicable Disease Report* (volume 8, no. 20, 15 May 1998). The tabulations for the first 19 weeks of the years 1997 and 1998 were averaged and then multiplied by 2.737 to extrapolate them to a full year.

strains isolated from human clinical specimens, plants, animals, and the environment. One example is the recent study of Müller et al. (77), who found six new species of *Buttiauxella* and one new species of *Kluyvera* in a large collection of strains isolated from snails. Clinical microbiologists should always be aware that most of the *Enterobacteriaceae* that they encounter every day will belong to just a few of the many species described. However, the expanding number of *Enterobacteriaceae* species is becoming a serious problem for reference laboratories and for commercial identification systems, whose identification methods are becoming inadequate for complete and accurate identification. Strains of *Enterobacteriaceae* isolated from plants, animals, and the environment can belong to any of the described species (Table 1), not just the species that were originally isolated from human clinical specimens. When a commercial identification gives an unusual organism for a final identification, there are several possibilities to consider: the identification is correct, just unusual; the identification is incorrect for a number of possible reasons; another aerobic or anaerobic organism is present and the biochemical profile is the result of all the metabolic activities of the mixture; or a handling or coding error was made somewhere along the way. Before a final report of an unusual organism is issued, it is advisable to do as much checking as possible. This could include repeating the biochemical tests in the same commercial system after confirming the absence of a contaminating aerobic or anaerobic organism; testing the isolate in another commercial identification system; and comparing the strain's antibiogram with known patterns reported for this organism. If these steps do not resolve the problem, the state health department can be contacted for advice, and the culture will often be accepted for further study. It is not uncommon for our reference laboratory to receive strains with a request such as: "Commercial identification system 1 called this species A, system 2 called it species B, and the state health department thinks it is neither of these but is species C. Please give us your opinion."

## Changes in Classification

There is no designated international body that considers all proposed changes in classification and then issues a formal ruling on whether to accept them. For many years there has been a Subcommittee on *Enterobacteriaceae* of the International Committee on Systematic Bacteriology, whose responsibility is the nomenclature and classification of *Enterobacteriaceae*. This Subcommittee can make recommendations on these matters but has rarely done so in the past. Even if this Subcommittee studies a specific change in classification, it can only make a recommendation, which can then be accepted or rejected by the scientific community. It should be emphasized that changes in classification are decided by usage not by judicial action. Sometimes two classifications are widely used, and both can be "correct." Classifications are correct if they conform to all the nomenclatural rules in the *Bacteriological Code* (*International Code of Nomenclature of Bacteria*), but they can be useful or not useful.

## Changes in Classification Incorporated in Table 1

Since the sixth edition of this Manual, some changes in classification have been proposed in the literature. Several of these appear to be clearly justified and have been incorporated in Table 1, but others have not been fully discussed or accepted by the scientific community. Table 1 gives the classification used by the Enteric Reference Laboratory, Foodborne and Diarrheal Diseases Laboratory Section, at the Centers for Disease Control and Prevention, which may differ from other classifications.

### *Xenorhabdus-Photorhabdus*

Based on ecological, phenotypic, and molecular evidence, Boemare et al. (9) proposed to reclassify the organism *Xenorhabdus luminescens* in a new genus, *Photorhabdus* as *Photorhabdus luminescens*. This change seems very logical and prudent because it separates *Xenorhabdus* and *Photorhabdus*, two genera that are phenotypically distinct and distantly related by DNA-DNA hybridization. In Table 1, the organism formerly known (36) as *Xenorhabdus luminescens* DNA hybridization group 5 is now classified as *Photorhabdus* DNA hybridization group 5. Clinical microbiologists should be aware of this organism because it occasionally occurs in human clinical specimens and has caused a few cases of bacteremia and wound infection (36).

TABLE 3 Important causes of nosocomial infections in the United States[a]

| Organism | No. (%) of isolates | | | | |
|---|---|---|---|---|---|
| | Urinary tract infection | Wound or surgical site | Pneumonia | Blood | Total |
| *Enterobacteriaceae* | | | | | |
| *Escherichia coli* | 20,218 (25.1) | 3,600 (9.2) | 1,607 (5.2) | 1,511 (5.2) | 27,871 (13.7) |
| *Enterobacter*, all species | 4,232 (5.2) | 2,850 (7.3) | 3,257 (10.6) | 1,316 (4.5) | 12,757 (6.2) |
| *Klebsiella pneumoniae* | 5,544 (6.9) | 1,250 (3.2) | 2,230 (7.2) | 1,280 (4.4) | 11,015 (5.4) |
| *Proteus mirabilis* | 4,077 (5.1) | 1,246 (3.2) | 779 (2.5) | 197 (0.7) | 4,662 (2.3) |
| *Citrobacter*, all species | 1,553 (1.9) | 598 (1.5) | 418 (1.4) | 174 (0.6) | 2,912 (1.4) |
| *Serratia marcescens* | 688 (0.9) | 548 (1.4) | 1,112 (3.6) | 351 (1.2) | 3,010 (1.5) |
| Other organisms for comparison | | | | | |
| Enterococci | 12,595 (15.6) | 4,998 (12.8) | 607 (2.0) | 2,594 (9.0) | 22,033 (10.8) |
| *Pseudomonas aeruginosa* | 9,309 (11.5) | 3,169 (8.1) | 5,162 (16.8) | 1,095 (3.8) | 20,307 (9.9) |
| *Staphylococcus aureus* | 1,569 (2.1) | 7,371 (18.8) | 5,352 (17.4) | 4,625 (16.0) | 23,187 (11.4) |
| Coagulase-negative staphylococci | 3,035 (3.8) | 5,147 (13.1) | 637 (2.1) | 8,481 (29.3) | 20,465 (10.0) |
| *Candida albicans* | 5,933 (7.4) | 984 (2.5) | 1,419 (4.6) | 1,380 (4.8) | 10,706 (5.2) |
| *Streptococcus*, all species | 1,265 (1.6) | 1,303 (3.3) | 1,050 (3.4) | 1,053 (3.6) | 4,998 (2.4) |
| *Candida*, other species | 1,763 (2.2) | 229 (0.6) | 245 (0.8) | 879 (3.0) | 3,370 (1.7) |

[a] The first figure is the number of isolates; the number in parentheses is the percentage. Based on nosocomial infection surveillance of over 200,000 cases for the United States, 1986 to 1989 (86) and 1990 to 1996 (unpublished data).

### *Citrobacter diversus-Citrobacter koseri*

The names *Citrobacter diversus* and *Citrobacter koseri* have both been used in the literature for some time, but the name *Citrobacter diversus* has been used much more frequently. Many workers recognized the phenotypic similarity of these two organisms and thought that they might be the same. The name *Citrobacter diversus* became the correct name for this organism on 1 January 1980, when the *Approved Lists of Bacterial Names* was issued, because under the laws of priority it was the older name. However, in 1993 the Judicial Commission of the International Committee on Systematic Bacteriology issued an Opinion (66) that the name *Citrobacter koseri* should be conserved over the name *Citrobacter diversus*, even though the name *Citrobacter diversus* was the older name, the correct name for the organism under the rules of the *Bacteriological Code*, and the name used most frequently in the literature. This "opinion" needs much more discussion by the scientific community, which is beyond the scope of this chapter; therefore, both names are included in Table 1.

## Other Proposed Changes in Nomenclature and Classification

### *Enterobacter agglomerans* Group-*Pantoea*

In 1972, Ewing and Fife (29) redefined the name *Enterobacter agglomerans* to include a wide variety of organisms known under many different names. These investigators also defined 11 different biogroups to recognize the phenotypic diversity of the many strains included in *Enterobacter agglomerans*. This name has become a useful one for clinical microbiologists and has been used extensively in the literature. Systematic analysis by Brenner and coworkers using DNA-DNA hybridization indicated that *Enterobacter agglomerans* is very heterogeneous, with at least 14 DNA hybridization groups (12). For this reason, the names "*Enterobacter agglomerans* complex" or "*Enterobacter agglomerans* group" (33) have been used to better indicate the heterogenicity of this "species." However, it has been very difficult to find simple tests to differentiate and identify all of the DNA hybridization groups (33). For this reason, workers have been reluctant to subdivide the *Enterobacter agglomerans* group until a definitive classification could be proposed (33). However, Gavini et al. (43) recently took the first step in a more logical classification for this complex group. They confirmed the close relatedness of a group of six strains that Brenner et al. had defined as "DNA hybridization group 13 of *Enterobacter agglomerans*" and proposed that this group be classified in a new genus, *Pantoea*. They also defined a new species in the genus, *Pantoea dispersa* (43), that corresponded to DNA hybridization group 3 of *Enterobacter agglomerans* of Brenner (12). The removal of these strains formerly classified in two DNA-DNA hybridization groups of *Enterobacter agglomerans* into a new genus, *Pantoea*, is a logical first attempt to reduce the heterogenicity of the genus *Enterobacter* and is based on sound scientific evidence.

However, this new classification has caused some problems. Some authors have broadened the original definition of Gavini et al. for *Pantoea agglomerans*. Gavini et al. were very careful to define *Pantoea agglomerans* to include only the strains that were closely related by DNA-DNA hybridization to strain ATCC 27155. Similarly, *Pantoea dispersa* was carefully defined to include only the strains that were highly related by DNA-DNA hybridization to the type strain of this new species, ATCC 14589. For the genus *Pantoea* and its species to remain homogeneous and well defined, these original species definitions must be adhered to very strictly. Since DNA-DNA hybridization is not routinely available and since simple tests are not available to definitively identify strains to the level of DNA hybridization group, it seems prudent to retain the vernacular name "*Enterobacter agglomerans* group" as a convenient name for clinical microbiologists for routine identification. This term is defined biochemically in Table 1, and it should be emphasized that it is used merely for convenience and because the name *Enterobacter agglomerans* is well understood and widely used in the literature. Eventually, this term will be replaced with a better classification. When definitive testing in a

TABLE 4 *Salmonella* and *Shigella* serotypes in the United States[a]

| Rank and serotype | No. of isolates |
|---|---|
| *Salmonella* serotypes | |
| 1 Enteritidis | 9,566 |
| 2 Typhimurium | 9,500 |
| 3 Heidelberg | 1,998 |
| 4 Newport | 1,985 |
| 5 Montevideo | 1,227 |
| 6 Javiana | 749 |
| 7 Oranienburg | 689 |
| 8 Hadar | 658 |
| 9 Agona | 606 |
| 10 Munchen | 595 |
| 11 Thompson | 587 |
| 12 Saintpaul | 562 |
| 13 Branderup | 532 |
| 14 Infantis | 503 |
| 15 Typhi | 440 |
| 16 Poona | 415 |
| 17 Paratyphi B | 337 |
| 18 Anatum | 270 |
| 19 Java | 250 |
| 20 Mbandaka | 223 |
| Subtotal (20 most common serotypes) | 31,692 |
| Other important (invasive) serotypes: | |
| Paratyphi A | 79 |
| Paratyphi C | 22 |
| Choleraesuis | 34 |
| Other serotypes, or not typed | 10,649 |
| Total *Salmonella* isolates | 34,520 |
| *Shigella* serotypes | |
| 1 *Shigella sonnei* (serogroup D) | 11,094 |
| 2 *Shigella flexneri* (serogroup B) | 4,015 |
| 3 *Shigella boydii* (serogroup C) | 396 |
| 4 *Shigella dysenteriae* (serogroup A) | 181 |
| *Shigella*, not completely typed | 1,573 |
| Total *Shigella* isolates | 17,259 |

[a] Data are from the latest published annual summaries from the Centers for Disease Control and Prevention. *Salmonella* Surveillance for 1996 and *Shigella* Surveillance for 1993 to 1995.

reference laboratory (usually including DNA hybridization) is done, more precise names can be used in reporting. Examples could include *Pantoea agglomerans* (DNA hybridization group 13), *Pantoea dispersa* (DNA hybridization group 3), *Enterobacter agglomerans* DNA hybridization group 1, *Enterobacter agglomerans* DNA hybridization group 2, *Enterobacter agglomerans* DNA hybridization group 12, etc. Precise identification to the level of DNA-DNA hybridization group is not available to the clinical microbiologist and will be limited to bacterial taxonomists and *Enterobacteriaceae* reference laboratories until simple identification methods become available.

### *Enterobacter taylorae-Enterobacter cancerogenus*

*Enterobacter taylorae* and *Enterobacter cancerogenus* may be two names for the same organism (44). However, they have different type strains; therefore, they are not "objective synonyms" under the rules of the *Bacteriological Code*. Until the identity of these two organisms is confirmed by other laboratories, both names will be used (Table 1).

## Nomenclature, Classification, and Reporting of the Genus *Salmonella*

After much study and discussion, there is now good agreement on most of the nomenclature and classification of the genus *Salmonella* (27, 37, 76, 81, 83). However, there are still several problem areas where different names or classifications are being used. These include the names *Salmonella choleraesuis* versus *Salmonella enterica*; the use of the terms "serotype" (sometimes abbreviated as ser.) versus "serovar"; the best way to write the names of the serotypes (serovars); the use of names versus antigenic formulas for some of the serotypes; and the argument whether some well-known serotype names should be eliminated and combined with other serotypes. Most of these points are discussed by McWhorter-Murlin and Hickman-Brenner (76), who summarize the differences between the nomenclature used at CDC (see chapter 28 for examples of this nomenclature and usage) and that used at the Institut Pasteur.

### Historical Species Concept in the Genus *Salmonella*

Until the 1970s, the species concept in the genus *Salmonella* was based on epidemiology, host range, biochemical reactions, and antigenic structure (the O antigen, phases 1 and 2 of the H antigen, and the Vi antigen, if present), and strains that differed in one or all of these properties were given distinct names. Names such as *Salmonella typhi*, *Salmonella choleraesuis* (originally some names were written with a hyphen, which was eventually dropped), *Salmonella paratyphi* A, *Salmonella paratyphi* A var. *durazzo*, *Salmonella typhimurium*, *Salmonella typhimurium* var. *copenhagen*, *Salmonella enteritidis*, and *Salmonella newport* began to appear, and the list rapidly expanded to hundreds of names. Some workers believed that these names really represented biological species (69), but others thought they were antigenic and biochemical varieties with an uncertain evolutionary relationship. However, there was universal agreement that the names were an extremely useful way to communicate about the particular serotypes. Most authors wrote the serotype names in italics as a species in the genus *Salmonella*, for example, *Salmonella typhimurium* (37).

### Basis for the Current Classification of the Genus *Salmonella*

In 1973, Crosa et al. (24) used DNA-DNA hybridization to show that *Salmonella* strains could be grouped into five main evolutionary groups. Two (possibly three) additional groups are now known (11, 81, 83). The vast majority of strains that cause infections in humans occurred in DNA hybridization group 1 (I). Strains isolated from animals and the environment clustered together into the four other groups, designated DNA groups 2 (II), 3a (IIIA), 3b (IIIB), and 4 (IV). Over the years, different authors have used different terms to refer to these evolutionary groups: DNA-DNA hybridization groups (24), multilocus enzyme electrophoresis clusters (11, 83), subgenera (69), species (see the *Approved Lists of Bacterial Names*), and subspecies (76, 81, 82).

### A Single Species of *Salmonella*—*Salmonella choleraesuis*

Crosa et al. (24) showed that all five groups of *Salmonella* were very highly related. With the species definition usually

used in DNA hybridization, these five groups could be considered as belonging to the same species. Under the rules of the *Bacteriological Code*, the name of this species is *Salmonella choleraesuis*. However, this species name can cause confusion, since *Salmonella choleraesuis* would have two totally different meanings, one as a species and one as a serotype.

### A Single Species of *Salmonella*—*Salmonella enterica*

There has been support for making an exception to the rules of the *Bacteriological Code* and using a name that would not cause confusion. There was a formal proposal to coin a new name, *Salmonella enterica*, that would replace the name *Salmonella choleraesuis* as the species name to represent most of the serotypes of *Salmonella*. The main advantage of this proposal is that it would reduce confusion by using a new name that has never been used as a serotype name. However, the proposal to change the name from *Salmonella choleraesuis* to *Salmonella enterica* was denied by the Judicial Commission of the International Committee on Systematic Bacteriology; hence, the name *Salmonella choleraesuis* remains the correct name. A second proposal to the Judicial Commission is being prepared; if approved, it would change the status of *Salmonella enterica* from "without standing in nomenclature, thus illegitimate" to "with standing in nomenclature, and legitimate." The name *Salmonella enterica* is being used by the World Health Organization's International Center for *Salmonella* (81) and by some of the World Health Organization's National Centers for *Salmonella*, including the one in the United States (76). The name is also appearing in the literature.

### Different Nomenclatures for Serotype Names

Another point of disagreement concerns the method of writing serotype names. For almost 100 years, serotype names have been written as species (the "serotype as species" nomenclature), and this method is still widely used. An example of this nomenclature: "*Salmonella enteritidis* is now the most common serotype in the United States and in many European countries."

Recently, the World Health Organization's International Center for *Salmonella*, a laboratory at the Institut Pasteur, Paris, France, introduced a different nomenclature in which the serotype name is capitalized and not written in italics. In this nomenclature, the name *Salmonella enteritidis* in the previous paragraph would be written in one of the following ways: "*Salmonella* serovar Enteriditis," "*Salmonella* ser. Enteritidis," or "*Salmonella* Enteritidis." The nomenclature described by McWhorter-Murlin and Hickman-Brenner (76) is similar, but they recommend using the term "serotype" instead of "serovar." The main advantage of these nomenclatures is that they do not artificially treat the serotypes as species. The main disadvantage is that they create a new nomenclature, which differs from one that has been widely accepted and used for many years, since there have been literally hundreds of thousands of uses of the "serotype as species" nomenclature in the literature. The International *Salmonella* Center's nomenclature is being used (often with modifications) by some of the National Centers for *Salmonella*, including the one at the Centers for Disease Control and Prevention (76). However, other National Centers for *Salmonella*, such as the one in England, continue to use the "serotype as species" nomenclature (for examples, see publications from the World Health Organization's National Center for *Salmonella* in England and epidemiological tabulations in the English epidemiological bulletin, *CDR Reports*). Since *Salmonella* names are being written differently by different authors and different National Centers for *Salmonella*, it is not surprising that the literature is beginning to reflect this variety of names. Recent examples of the way "serotype Typhimurium" is being written include *Salmonella* serotype Typhimurium, *Salmonella* ser. Typhimurium, *Salmonella typhimurium*, *Salmonella* Typhimurium, *Salmonella* typhimurium, *Salmonella* serovar Typhimurium, and *Salmonella* serovar typhimurium. When the above variations are combined with the four species/subspecies possibilities, i.e., *Salmonella choleraesuis*, *Salmonella choleraesuis* subspecies *choleraesuis*, *Salmonella enterica*, and *Salmonella enterica* subspecies *enterica*, the number of possible variations is multiplied considerably (one example of the over two dozen possible ones is *Salmonella enterica* subspecies *enterica* serovar Typhimurium).

### Laboratory Reports for Isolates of *Salmonella*

Most clinical microbiology laboratories will identify a *Salmonella* isolate with a commercial identification system and then usually with *Salmonella* antisera. These two methods usually give definitive results, and a report can be issued such as "*Salmonella* serogroup B." Thus, fortunately, clinical microbiology laboratories are usually immune from the above problems in their reporting. A reference laboratory can do definitive serotyping and biochemical testing and can determine a complete serotype. A report such as "*Salmonella* serotype Typhimurium" can be issued. The advantage of this simple wording of reports is that it avoids the subgenus/species/subspecies concept entirely and concentrates on the actual laboratory results, which should be easily understood.

## Nomenclature for Shiga Toxins/Verotoxins Produced by *E. coli* and *Shigella*

In a similar vein to the problems described for *Salmonella*, several different names are being used in the literature for the cytotoxins produced by *E. coli* and *Shigella*. This topic is critical because of the importance of *E. coli* O157 and other strains that produce these toxins (see chapter 28). Several different commercial assays for these toxins are being marketed; thus it is essential to read the package insert carefully to determine exactly which toxin(s) the kit is detecting, and to word laboratory reports accordingly.

For almost 100 years, it has been known that *Shigella dysenteriae* O1 produces a potent cytotoxin known as Shiga toxin. More recently, it has been shown that certain diarrheagenic strains of *E. coli* produced a similar toxin that was first detected because it was cytotoxic for Vero cells in tissue culture. A number of recent studies have defined these proteins from *Shigella dysenteriae* O1 and *E. coli*, and there is agreement that they comprise a family of toxins. They are being referred to in the literature as Shiga toxin, Shiga-like toxins, and verotoxins; and at least five different toxins are involved (20). The main disagreement in the literature is that some workers have referred to these cytotoxins produced by *E. coli* as "Shiga-like toxins," but others have used the term "verotoxins" (VT). Recently, Calderwood et al. summarized the data available and proposed a new nomenclature for the toxins and for their corresponding genes (20). They recommended that strains of *E. coli* that produce these toxins be called "Shiga toxin-producing" *E. coli*, which would replace the previous term, "Shiga-like toxin producing." They also recommended that the new toxin name be cross-referenced with the corresponding verotoxin name. With this nomenclature, a laboratory report for a stool cul-

ture might be worded, "positive for *E. coli* O157:H7, which produces Shiga toxins Stx1 (VT1) and Stx2 (VT2)." Hopefully the differences between the groups using the two different nomenclatures can be resolved, resulting in a single nomenclature in the literature.

## DESCRIPTION OF THE FAMILY *ENTEROBACTERIACEAE*

Most organisms in the family *Enterobacteriaceae* share the following properties: they are gram negative and rod shaped; do not form spores; are motile by peritrichous flagella or nonmotile; grow on peptone or meat extract media without the addition of sodium chloride or other supplements; grow well on MacConkey agar; grow both aerobically and anaerobically; are active biochemically; ferment (rather than oxidize) D-glucose and other sugars, often with gas production; are catalase positive and oxidase negative; reduce nitrate to nitrite; contain the enterobacterial common antigen; and have a 39 to 59% guanine-plus-cytosine (G + C) content of DNA (4). Species in the family should also be more closely related (by techniques that measure evolutionary distance) to *Escherichia coli*, the type species of the type genus of the family, than they are to organisms in other families.

## NATURAL HABITATS

*Enterobacteriaceae* are widely distributed on plants and in soil, water, and the intestines of humans and animals (4). Some species occupy very limited ecological niches. *Salmonella typhi* causes typhoid fever and is found only in humans (53). In contrast, strains of *Klebsiella pneumoniae* are distributed widely in the environment and contribute to biochemical and geochemical processes (71). However, strains of *K. pneumoniae* also cause human infections, ranging from asymptomatic colonization of the intestinal, urinary, and respiratory tracts to fatal pneumonia, septicemia, and meningitis.

## CLINICAL SIGNIFICANCE

Strains of *Enterobacteriaceae* are associated with abscesses, pneumonia, meningitis, septicemia, and infections of wounds, the urinary tract, and the intestines. They are a major component of the normal intestinal flora of humans but are relatively uncommon at other body sites. Several species of *Enterobacteriaceae* are very important causes of nosocomial infections (Table 3). *Enterobacteriaceae* may account for 80% of clinically significant isolates of gram-negative bacilli and 50% of clinically significant bacteria in clinical microbiology laboratories. They account for nearly 50% of septicemia cases (Table 2), more than 70% of urinary tract infections, and a significant percentage of intestinal infections.

### Human Extraintestinal Infections

Except for the species of *Shigella*, which rarely cause infections outside the gastrointestinal tract, many species of *Enterobacteriaceae* commonly cause extraintestinal infections. However, a small number of species, i.e., *E. coli*, *Klebsiella pneumoniae*, *K. oxytoca*, *Proteus mirabilis*, *Enterobacter aerogenes*, *Enterobacter cloacae*, and *Serratia marcescens*, account for most of these infections (Tables 2 and 3). Urinary tract infections, primarily cystitis, are the most common (86), followed by respiratory, wound, bloodstream, and central nervous system infections. Many of these infections, especially sepsis and meningitis, are life-threatening and are often hospital acquired. Because of the severity of these infections, prompt isolation, identification, and susceptibility testing of *Enterobacteriaceae* isolates are essential.

### Human Intestinal Infections

Several organisms in the family *Enterobacteriaceae* are also important causes of intestinal infections of humans (Table 4) and animals worldwide. Although other species in the family have been associated with diarrhea (91) or even implicated as causes of diarrhea, only organisms in four genera, *Escherichia* (26, 32, 68, 93), *Salmonella* (24, 37, 53, 81), *Shigella* (27), and *Yersinia* (6, 67, 84), have been clearly documented as enteric pathogens. These four genera are discussed in chapters 28 and 30. Other *Enterobacteriaceae* such as *Citrobacter*, *Edwardsiella*, *Hafnia*, *Morganella*, *Proteus*, *Klebsiella*, *Enterobacter*, and *Serratia*, have occasionally been implicated (4, 91). Strains that produce biologically active compounds (these are often overstated as being "enterotoxin-producing strains") of these *Enterobacteriaceae* have been isolated from people with diarrhea (91), but the etiological roles of these strains are uncertain. Some laboratories issue reports for stool cultures such as, "*Klebsiella pneumoniae* isolated in essentially pure culture (10 of 10 colonies tested); please consult the laboratory to discuss possible significance," to reflect this drastic change in the stool flora. There is no evidence that strains of these other genera are important causes of diarrhea.

## SPECIMEN COLLECTION, TRANSPORT, AND PROCESSING

### Extraintestinal Specimens

*Enterobacteriaceae* are recovered from infections at many different body sites, and normal practices (see chapters 4 and 5) for collecting blood, respiratory, wound, urine, and other specimens should be followed.

### Intestinal Specimens

Stool cultures are usually submitted to the laboratory with a request to isolate and identify the cause of a possible intestinal infection, usually manifested as diarrhea. The groups of *Enterobacteriaceae* usually associated with diarrhea in the United States are *Salmonella*, *Shigella*, and certain pathogenic strains of *E. coli* and *Yersinia enterocolitica*.

Stool specimens require special attention to both collection and transportation and should be obtained early in the course of illness, when the causative agent is likely to be present on primary plates in the largest numbers. At this stage, the use of enrichment broths should be unnecessary. Except for typhoid fever, the isolation rate for enteric pathogens declines as the patient recovers. Freshly passed stool is a better specimen than rectal swabs, since there is less chance for improper collection and since mucus and blood-stained portions can be selected for culture. Stool cultures should be plated within 2 h of collection, to recover fastidious pathogens such as *Shigella*. If rapid processing is not possible, a small portion of feces or a swab coated with feces should be placed in transport medium. Commonly used transport media include Stuart, Amies, Cary-Blair, and buffered glycerol saline. Cary-Blair is probably the best overall transport medium for diarrheal stools. Specimens held in transport media should be refrigerated until examined. In certain circumstances, such as with infants or during mass

screening in outbreak investigations, rectal swabs may be useful. Mucosa within the rectal vault must be sampled for these specimens to be of optimum value. Many different procedures have been described for processing stool specimens. In cases of chronic or severe diarrhea, rare pathogens should also be considered. More information about the isolation, identification, typing, and virulence testing of isolates of *Salmonella*, *Shigella*, *E. coli*, and *Y. enterocolitica* is given in chapters 28 and 30.

### Microscopic Examination

Stool specimens should be examined visually for the presence of blood or mucus, but microscopic examination should not be done routinely because of its lack of specificity (22, 26, 51, 80). Although identification by fluorescent-antibody staining is theoretically possible for all enteric pathogens, it has been of limited success because the method is difficult and there are many serological cross-reactions among the species of *Enterobacteriaceae* (27). This technique has been limited to detection of *Salmonella* strains (primarily by the food industry) and certain serogroups of *E. coli* and to outbreak investigations.

## ISOLATION

### Extraintestinal Specimens

Most strains of *Enterobacteriaceae* grow readily on the plating media commonly used in clinical microbiology laboratories. Specimens from body sites that are normally sterile are cultured on blood or chocolate agar. Urine, respiratory tract, and wound specimens, which are likely to contain mixtures of organisms, are almost always cultured on selective media to enhance the recovery of *Enterobacteriaceae*. MacConkey agar, generally interchangeable with eosin methylene blue (EMB) agar, is usually used, because it allows a preliminary grouping of enteric and other gram-negative bacteria. The most common isolates of *Enterobacteriaceae* have a characteristic appearance on blood agar and MacConkey agar that is useful for preliminary identification (Table 5). Broth enrichment can increase the isolation rate if small numbers of *Enterobacteriaceae* are present, but this step is not normally required.

### Intestinal Specimens

Media that should be used routinely for intestinal specimens include a nonselective medium such as blood agar, a differential medium of low to moderate selectivity such as MacConkey agar, and a more selective differential medium such as xylose-lysine-deoxycholate (XLD) agar or Hektoen enteric (HE) agar. A broth enrichment such as selenite (or GN or tetrathionate) can be included, particularly if the specimen is not optimal. A highly selective medium such as brilliant green agar or bismuth sulfite agar can also be included for isolating strains of *Salmonella*. A plate of sorbitol-MacConkey (SMAC) agar can be added to enhance the isolation of Shiga toxin-producing strains of *E. coli* O157:H7. This medium should be used if the stool is frankly bloody or if the patient has a diagnosis of hemolytic-uremic syndrome (HUS), and it can be used for all fecal specimens if resources permit (see chapter 28). When *Y. enterocolitica* is suspected, a selective-differential medium, such as CIN (cefsulodin-irgasan-novobiocin) agar (also called *Yersinia* selective agar), can be added (see chapter 30). A complete stool culture procedure should also include media for isolation of *Campylobacter* and possibly *Vibrio* strains in areas where cholera and other *Vibrio* infections are common.

## IDENTIFICATION

There are many different approaches to identifying strains of *Enterobacteriaceae* (33).

### Conventional Biochemical Tests in Tubes

Tube testing was once used by all clinical microbiology laboratories, and it is still widely used in reference and public health laboratories (27, 33). Although many laboratories prepare their own media from commercial dehydrated powders, most of the common media are also available commercially in glass tubes that are ready to inoculate. Growth from a single colony is inoculated into each tube, and the tests are read at 24 h and usually also at 48 h. In the Enteric Reference Laboratory, most tests are read for 7 days. Unfortunately, the media and tests are not completely standardized, and few laboratories use exactly the same formulations or procedures. Even with these variables, this approach usually results in correct identifications of the common species of *Enterobacteriaceae*. Table 1 gives the results for *Enterobacteriaceae* in 47 tests (for the media and methods used to generate the data in this table, see references 31 and 53).

### Computer Analysis To Assist in Identification

Two microcomputer programs have been developed in the Enteric Reference Laboratory to assist with the identifica-

TABLE 5 Colonial appearance of some of the most common *Enterobacteriaceae* on MacConkey agar and sheep blood agar[a]

| Genus or species | Appearance and typical colony diameter on: | |
|---|---|---|
| | MacConkey agar | Sheep blood agar[b] |
| *Salmonella* and *Shigella* | Colorless, flat, 2–3 mm | Smooth, 2–3 mm |
| *Yersinia enterocolitica* | Colorless, <1 mm | Smooth, <1 mm |
| *Escherichia coli* (lactose positive) | Red, usually surrounded by precipitated bile, 2–3 mm | Smooth, 2–3 mm |
| *Escherichia coli* (lactose negative) | Colorless, 2–3 mm | Smooth, 2–3 mm |
| *Klebsiella pneumoniae* | Pink, mucoid, 3–4 mm | Mucoid, 3–4 mm |
| *Enterobacter* | Pink, not as mucoid as *Klebsiella*, 2–4 mm | Smooth, 3–4 mm |
| *Proteus vulgaris* and *Proteus mirabilis* | Colorless, flat, often swarm slightly, 2–3 mm | Swarm in waves to cover plate |
| Other *Proteus*, *Providencia*, and *Morganella* species | Colorless, flat, no swarming, 2–3 mm | Flat, 2–3 mm, no swarming |

[a] Most strains appear this way, but there are exceptions.
[b] Unlike *Vibrionaceae*, most strains of *Enterobacteriaceae* are nonhemolytic. However, a few strains of *E. coli* are strongly hemolytic.

tion of *Enterobacteriaceae* cultures. "George" and "Strain matcher" were described in the 1985 review of the family (33). A detailed description and information for obtaining them is available by contacting the author and is being prepared for the Internet.

## Screening Tests, Using All Information Available

Over the years, the *Enterobacteriaceae* reference laboratories have found that many genera, species, and serotypes can be tentatively identified by a minimum number of screening tests (Table 6). These tests may be more useful in a reference or research laboratory that does not have to deal with all of the regulatory aspects of testing human clinical specimens. More precise identification can be made by using more tests or commercial identification systems. For example, a blood isolate has the following properties: colonies on MacConkey agar are 2 to 3 mm in diameter, are bright red and nonmucoid, and have precipitated bile around them; they are indole positive and 4-methylumbelliferyl-β-D-glucuronidase (MUG) positive; they grow at 44.5°C; and they are antibiotic resistant. These results are completely compatible with *Escherichia coli*. Similarly, an isolate from the feces of a diarrhea patient has the following properties: colonies on MacConkey agar are 2 to 3 mm in diameter and colorless; colonies on XLD agar are 2 to 3 mm and black; the isolate agglutinates in *Salmonella* polyvalent serum and in O-group

TABLE 6 Screening tests for genera and species of *Enterobacteriaceae* often isolated from human clinical specimens[a]

| Organism (genus, species, or serotype) | Test or property[b] |
|---|---|
| *Citrobacter* | Citrate$^+$, lysine decarboxylase$^-$, often grows on CIN agar, strong characteristic odor |
| *Enterobacter* | Variable biochemically, citrate$^+$, VP$^+$, resistant to cephalothin |
| *Escherichia coli* | Extremely variable biochemically, indole$^+$, MUG$^+$, grows at 44.5°C, sometimes antibiotic resistant, molecular test: PhoE$^{+d}$ |
| *Escherichia coli* O157:H7 | Colorless colonies on sorbitol-MacConkey agar, MUG$^-$, D-sorbitol$^-$ (or delayed), agglutinates in O157 serum and H7 serum |
| *Hafnia* | Lysed by *Hafnia* specific bacteriophage[c]; often more active biochemically at 25 than 36°C |
| *Klebsiella* | Mucoid colonies, encapsulated cells, nonmotile, lysine$^+$, very active biochemically, ferment most sugars, VP$^+$, malonate$^+$, resistant to carbenicillin and ampicillin |
| *Proteus-Providencia-Morganella* | Phenylalanine$^+$, tyrosine hydrolysis$^+$, often urea$^+$, resistant to colistin |
| *Morganella* | Very inactive biochemically, no swarming, citrate$^-$, $H_2S^-$, ornithine$^+$, gelatin$^-$, lipase$^-$, urea$^+$ |
| *Proteus* | Swarms on blood agar, pungent odor, $H_2S^+$, gelatin$^+$, lipase$^+$ |
| *Proteus mirabilis* | Urea$^+$, indole$^-$, ornithine$^+$, maltose$^-$ |
| *Proteus vulgaris* | Urea$^+$, indole$^+$, ornithine$^-$, maltose$^+$ |
| *Providencia* | No swarming, $H_2S^-$, ornithine$^-$, gelatin$^-$, lipase$^-$ |
| *Salmonella* | Lactose$^-$, sucrose$^-$, $H_2S^+$, O1 phage$^{+c}$, MUCAP$^{+d}$, agglutinates in polyvalent serum[b], typical colonies on media selective or differential for *Salmonella* (brilliant green agar, SS agar, Rambach agar, etc.), lysed by *Salmonella*-specific bacteriophage,[c] often antibiotic resistant |
| *Salmonella typhi* | $H_2S^+$ (trace amount only), agglutinates in group D serum |
| *Serratia* | DNase$^+$, gelatinase$^+$, lipase$^+$, resistant to colistin and cephalothin |
| *Serratia marcescens* | L-Arabinose$^-$ |
| *Serratia*, other species | L-Arabinose$^+$ |
| *Shigella* | Nonmotile, lysine$^-$, gas$^-$, agglutinates in polyvalent serum, biochemically inactive, often antibiotic resistant, molecular test: PhoE$^{+d}$ |
| *Shigella boydii* | Agglutinates in group C serum, D-mannitol$^+$ |
| *Shigella dysenteriae* | Agglutinates in group A serum, D-mannitol$^-$ |
| *Shigella dysenteriae* O1 | Catalase$^-$, agglutinates in O1 serum, Shiga toxin$^+$ |
| *Shigella flexneri* | Agglutinates in group B serum, D-mannitol$^+$ |
| *Shigella sonnei* | Agglutinates in group D serum, D-mannitol$^+$, ornithine decarboxylase$^+$, lactose$^+$ (delayed), colony variation: smooth to rough |
| *Yersinia* | Grows on CIN agar, often more active biochemically at 25 than 36°C (motile at 25°C, nonmotile at 36°C), urea$^+$ |
| *Yersinia enterocolitica*, pathogenic serotypes | CR-MOX$^+$, pyrazinamidase$^-$, salicin$^-$, esculin$^-$, agglutinates in O sera: 3; 4,32; 5,27; 8; 9; 13a,13b; 18; 20; or 21 |
| *Yersinia enterocolitica* O3 (a pathogenic serotype) | D-Xylose$^-$, agglutinates in O3 serum, tiny colonies at 24 h on plating media |
| *Yersinia enterocolitica*, nonpathogenic serotypes | CR-MOX$^-$, pyrazinamidase$^+$, salicin$^+$, esculin$^+$, no agglutination in O sera: 3; 4,32; 5,27; 8; 09; 13a,13b; 18; 20; and 21 |

[a] This table gives only the general properties of the genera, species, serogroups, so there will be exceptions. See Table 1 and chapters 28 to 30 for more details and more precise data. The properties listed for a genus or group of genera generally apply for each of its species, and the properties listed for a species generally apply for each of its serotypes.

[b] Biochemical test results are given as percentages in Table 1. The serologic tests refer to slide agglutination in group or individual antisera for *Salmonella*, *Shigella*, *Yersinia*, or *E. coli*.

[c] These are two bacteriophage tests useful for identification.

[d] Abbreviations: CIN, cefsulodin-irgasan-novobiocin agar (a plating medium selective for *Yersinia*); CR-MOX, Congo red, magnesium oxalate agar (a differential medium useful for distinguishing pathogenic from nonpathogenic strains of *Yersinia*); MUCAP, 4-methylumbelliferyl caprylate (a genus-specific test for *Salmonella*); MUG, 4-methylumbelliferyl-β-D-glucuronidase; ONPG, *o*-nitrophenyl-β-D-galactopyranoside; PhoE, a test done by PCR that is sensitive and specific for *E. coli* and *Shigella* (see the text); VP, Voges-Proskauer.

B serum; the 4-methylumbelliferyl caprylate (MUCAP) test is positive; lysis by bacteriophage O1 is positive; and it is antibiotic resistant. All these results are compatible with *Salmonella* serogroup B.

### "Kits" for Identification

A kit is defined as a series of miniaturized or standardized tests that are available commercially. The approach in using kits is similar to the conventional tube method, with the main differences being in the miniaturization, number of tests available, suspending medium, and method of reading and interpreting results (sometimes by machine). Kits are now used by most American laboratories; they are discussed in chapter 11. Kits usually give the correct identification for the most common species of *Enterobacteriaceae*, but they may not be as accurate for some of the new species. It is important to check the instruction manual to determine which organisms are included in the database and the number of strains that were used to define each organism. The main problem with kit-based identification is that the tests used (usually about 20 tests) are becoming inadequate to differentiate all of the current species of *Enterobacteriaceae* given in Table 1. This is also a problem with conventional tube tests when an inadequate number is used. Unusual identifications or "no identification" obtained with a kit should be checked by other methods.

### Molecular Methods of Identification

Molecular methods have proved useful for identification to the level of family, genus, species, serotype, clone, and strain and for differentiation of pathogenic from nonpathogenic strains (see chapter 13). For example, a PCR test for the *phoE* gene appears to be a sensitive and specific test for determining if a culture belongs to *Escherichia-Shigella* (87). However, few if any of these molecular methods are commercially available. Commercial methods also must be approved by the Food and Drug Administration for use on human clinical specimens in the United States. These problems have greatly restricted the use of molecular methods for *Enterobacteriaceae* in clinical microbiology laboratories. However, they have proved extremely useful in a research setting. To conform with the CLIA (Clinical Laboratory Improvement Amendments of 1988, also called CLIA '88) regulations, it is necessary to report these research results with a disclaimer unless all the CLIA requirements have been met.

### Problem Strains

Most strains of *Enterobacteriaceae* grow rapidly on plating media and on media used for biochemical identification, but occasionally a slow-growing or fastidious strain is encountered. Some strains grow poorly on blood agar but much better on chocolate agar incubated in a candle jar. This characteristic suggests a possible nutritional requirement or a mutation involving respiration. There are slow-growing strains of *E. coli*, *K. pneumoniae*, and *Serratia marcescens*, and typical biochemical reactions of these strains usually require extended incubation. Another type of problem organism is sometimes isolated from patients who are taking antimicrobial agents. Li et al. described such "pleiotropic" (having multiple phenotypic expression) mutants of *S. marcescens* (74) and *Salmonella* after exposure to gentamicin. These strains react atypically in many of the standard biochemical tests and are difficult to identify. A different type of pleiotropic mutant induced by chemical exposure was reported by Lannigan and Hussian (72). A *Salmonella* strain lost the ability to produce hydrogen sulfide, reduce nitrate to nitrite, and produce gas from glucose because of chlorate resistance acquired after exposure to Dakin's solution (a solution that contains chlorate and is found in hospitals). Some atypical and slow-growing strains become more typical and grow better when they are transferred several times. Laboratories occasionally isolate strains that grow rapidly but do not have biochemical reactions that fit any of the described species, biogroups, or Enteric Groups of *Enterobacteriaceae*. At present, this type of culture can only be reported as "unidentified." It may be an atypical strain of one of the organisms listed in Table 1, or it may belong to a new species that has not been described. Additional testing at a state, national, or international reference laboratory can often answer this question, and has in the past led to the discovery of new causes of human infections (12, 16, 33, 77).

## ANTIBIOTIC SUSCEPTIBILITY

Several methods are available for testing the antibiotic susceptibility of *Enterobacteriaceae*, but the most popular are disk diffusion (5) and broth dilution (see chapter 118). In addition, the reader should consult a current textbook or review of infectious diseases for a description of antibiotic usage in clinical practice.

When antibiotics were first introduced, there was only slight resistance among the species of *Enterobacteriaceae*. Today, antibiotic resistance is much more common among strains isolated from humans and animals. Resistance patterns vary depending on the organism and its origin.

### Intrinsic Resistance

Intrinsic resistance is a genetic property of most strains of a species and evolved long before the clinical use of antibiotics. This evolution can best be shown by studying strains isolated and stored before the antibiotic era or by studying strains from nature that presumably have had less exposure to antibiotics. For example, essentially all strains of *Serratia marcescens* have intrinsic resistance to penicillin G, colistin, and cephalothin. Table 7 lists some species of *Enterobacteriaceae* and their intrinsic resistance patterns.

### The Antibiogram as a Marker in Epidemiological Studies

Antibiotic susceptibility testing is usually done on isolates that are clinically significant (see chapter 7) and provides an "antibiogram" that is useful for comparing isolates in epidemiologic studies. When the selective ecological pressure of antibiotics is changed, the resistance patterns of epidemic (or endemic) strains may also change. These changes have been documented in outbreaks that have lasted for several months or longer. Even with these limitations in stability, the antibiogram is probably the most useful and practical laboratory marker for comparing strains and can be extremely helpful in recognizing infection problems.

### Use of Antibiograms for Identification

The antibiogram of a culture can be compared with those of known isolates (Table 7) to provide a different approach to identification. When the antibiogram and identification are incompatible (for example, a culture of *Klebsiella* that is susceptible to ampicillin and carbenicillin, or a culture of *Enterobacter* that is susceptible to cephalothin), the culture should be streaked and checked for purity. In addition, both the identification and the antibiogram may have to be repeated.

TABLE 7 Intrinsic antimicrobial resistance in some of the common *Enterobacteriaceae*

| Genus or species | Most strains are resistant to: |
|---|---|
| *Buttiauxella* species | Cephalothin |
| *Cedecea* species | Polymyxins, ampicillin, cephalothin |
| *Citrobacter amalonaticus* | Ampicillin |
| *Citrobacter freundii* | Cephalothin |
| *Citrobacter diversus* | Cephalothin, carbenicillin |
| *Edwardsiella tarda* | Colistin |
| *Enterobacter cloacae* | Cephalothin |
| *Enterobacter aerogenes* | Cephalothin |
| Many other *Enterobacter* species | Cephalothin |
| *Escherichia hermannii* | Ampicillin, carbenicillin |
| *Ewingella americana* | Cephalothin |
| *Hafnia alvei* | Cephalothin |
| *Klebsiella pneumoniae* | Ampicillin, carbenicillin |
| *Kluyvera ascorbata* | Ampicillin |
| *Kluyvera cryocrescens* | Ampicillin |
| *Proteus mirabilis* | Polymyxins, tetracycline, nitrofurantoin |
| *Proteus vulgaris* | Polymyxins, ampicillin, nitrofurantoin, tetracycline |
| *Morganella morganii* | Polymyxins, ampicillin, cephalothin |
| *Providencia rettgeri* | Polymyxins, cephalothin, nitrofurantoin, tetracycline |
| Other *Providencia* species[a] | Polymyxins, nitrofurantoin |
| *Serratia marcescens*[b] | Polymyxins, cephalothin, nitrofurantoin |
| *Serratia fonticola* | Ampicillin, carbenicillin, cephalothin |
| Other *Serratia* species | Polymyxins,[c] cephalothin |

[a] Most strains of *Providencia stuartii* are also resistant to cephalothin and tetracycline.

[b] *Serratia marcescens* can also be resistant to ampicillin, carbenicillin, streptomycin, and tetracycline.

[c] Resistance to polymyxins is common in *Serratia* species, but some strains have zones of 10 to 12 mm or larger.

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## REFERENCES

1. **Aldová, E., O. Hausner, D. J. Brenner, Z. Kocmoud, J. Schindler, B. Potužníková, and P. Petráš.** 1988. *Pragia fontium* gen. nov., sp. nov. of the family *Enterobacteriaceae*, isolated from water. *Int. J. Syst. Bacteriol.* **38**:183–189.
2. **Aleksić, S., A. G. Steigerwalt, J. Bockemühl, G. P. Huntley-Carter, and D. J. Brenner.** 1987. *Yersinia rohdei* sp. nov. isolated from human and dog feces and surface water. *Int. J. Syst. Bacteriol.* **37**:327–332.
3. **Bagley, S. T., R. J. Seidler, and D. J. Brenner.** 1981. *Klebsiella planticola* sp. nov.: a new species of *Enterobacteriaceae* found primarily in nonclinical environments. *Curr. Microbiol.* **6**:105–109.
4. **Balows, A., H. G. Trüper, M. Dworkin, W. Harder, and K.-H. Schleifer (ed.).** 1992. *The Prokaryotes*, 2nd ed, vol. 3, p. 2673–2937. Springer-Verlag KG, Berlin, Germany.
5. **Bauer, A. W., W. M. M. Kirby, J. C. Sherris, and M. Turck.** 1966. Antibiotic susceptibility testing by a standardized single disk method. *Am. J. Clin. Pathol.* **45**: 493–496.
6. **Bercovier, H., D. J. Brenner, J. Ursing, A. G. Steigerwalt, G. R. Fanning, J. M. Alonso, G. A. Carter, and H. H. Mollaret.** 1980. Characterization of *Yersinia enterocolitica sensu stricto*. *Curr. Microbiol.* **4**:201–206.
7. **Bercovier, H., A. G. Steigerwalt, A. Guiyoule, G. Huntley-Carter, and D. J. Brenner.** 1984. *Yersinia aldovae* (formerly *Yersinia enterocolitica*-like group X2): a new species of *Enterobacteriaceae* isolated from aquatic ecosystems. *Int. J. Syst. Bacteriol.* **34**:166–172.
8. **Bercovier, H., J. Ursing, D. J. Brenner, A. G. Steigerwalt, G. R. Fanning, G. P. Carter, and H. H. Mollaret.** 1980. *Yersinia kristensenii*: a new species of *Enterobacteriaceae* composed of sucrose-negative strains (formerly called *Yersinia enterocolitica* or *Yersinia enterocolitica*-like). *Curr. Microbiol.* **4**:219–224.
9. **Boemare, N. E., R. J. Akhurst, and R. G. Mourant.** 1993. DNA relatedness between *Xenorhabdus* spp. (*Enterobacteriaceae*), symbiotic bacteria of entomopathogenic nematodes, and a proposal to transfer *Xenorhabdus luminescens* to a new genus, *Photorhabdus* gen. nov. *Int. J. Syst. Bacteriol.* **43**:249–255.
10. **Bouvet, O. M. M., P. A. D. Grimont, C. Richard, E. Aldova, O. Hausner, and M. Gabrhelova.** 1985. *Budvicia aquatica* gen. nov., sp. nov.: a hydrogen sulfide-producing member of the *Enterobacteriaceae*. *Int. J. Syst. Bacteriol.* **35**: 60–64.
11. **Boyd, E. F., F.-S. Wang, T. S. Whittam, and R. K. Selander.** 1996. Molecular genetic relationships of the salmonellae. *Appl. Environ. Microbiol.* **62**:804–808.
12. **Brenner, D. J.** 1992. Additional genera of *Enterobacteriaceae*, p. 2922–2937. *In* A. Balows, H. G. Trüper, M. Dworkin, W. Harder, and K.-H. Schleifer (ed.), *The Prokaryotes*, 2nd ed. Springer-Verlag KG, Berlin, Germany.
13. **Brenner, D. J.** 1992. Introduction to the family *Enterobacteriaceae*, p. 2673–2695. *In* A. Balows, H. G. Trüper, M. Dworkin, W. Harder, and K.-H. Schleifer (ed.), *The Prokaryotes*, 2nd ed. Springer-Verlag KG, Berlin, Germany.
14. **Brenner, D. J., H. Bercovier, J. Ursing, J. M. Alonso, A. G. Steigerwalt, G. R. Fanning, G. P. Carter, and H. H. Mollaret.** 1980. *Yersinia intermedia*: a new species of *Enterobacteriaceae* composed of rhamnose-positive, melibiose-positive, raffinose-positive strains (formerly called atypical *Yersinia enterocolitica* or *Yersinia enterocolitica*-like). *Curr. Microbiol.* **4**:207–212.
15. **Brenner, D. J., B. R. Davis, A. G. Steigerwalt, C. F. Riddle, A. C. McWhorter, S. D. Allen, J. J. Farmer III, Y. Saitoh, and G. R. Fanning.** 1982. Atypical biogroups of *Escherichia coli* found in clinical specimens and description of *Escherichia hermannii* sp. nov. *J. Clin. Microbiol.* **15**: 703–713.
16. **Brenner, D. J., P. A. D. Grimont, A. G. Steigerwalt, G. R. Fanning, E. Ageron, and C. F. Riddle.** 1993. Classification of citrobacteria by DNA hybridization: designation of *Citrobacter farmeri* sp. nov., *Citrobacter youngae* sp. nov., *Citrobacter braakii* sp. nov., *Citrobacter werkmanii* sp. nov., *Citrobacter sedlakii* sp. nov., and three unnamed *Citrobacter* genomospecies. *Int. J. Syst. Bacteriol.* **43**:645–658.
17. **Brenner, D. J., A. C. McWhorter, A. Kai, A. G. Steigerwalt, and J. J. Farmer III.** 1986. *Enterobacter asburiae* sp. nov., a new species found in clinical specimens, and reassignment of *Erwinia dissolvens* and *Erwinia nimipressuralis* to the genus *Enterobacter* as *Enterobacter dissolvens* comb.

nov. and *Enterobacter nimipressuralis* comb. nov. *J. Clin. Microbiol.* 23:1114–1120.

18. **Brenner, D. J., A. C. McWhorter, J. K. Leete Knutson, and A. G. Steigerwalt.** 1982. *Escherichia vulneris*: a new species of *Enterobacteriaceae* associated with human wounds. *J. Clin. Microbiol.* 15:1133–1140.
19. **Brenner, D. J., C. Richard, A. G. Steigerwalt, M. A. Asbury, and M. Mandel.** 1980. *Enterobacter gergoviae* sp. nov.: a new species of *Enterobacteriaceae* found in clinical specimens and the environment. *Int. J. Syst. Bacteriol.* 30: 1–6.
20. **Calderwood, S. B., D. W. K. Acheson, G. T. Keusch, T. J. Barrett, P. M. Griffin, N. A. Strockbine, B. Swaminathan, J. B. Kaper, M. M. Levine, B. S. Kaplan, H. Karch, A. D. O'Brien, T. G. Obrig, Y. Takeda, P. I. Tarr, and I. K. Wachsmuth.** 1996. Proposed new nomenclature for SLT (VT) family. *ASM News* 62:118–119.
21. **Chung, Y. R., D. J. Brenner, A. G. Steigerwalt, B. S. Kim, H. T. Kim, and K. Y. Cho.** 1993. *Enterobacter pyrinus* sp. nov., an organism associated with brown leaf spot disease of pear trees. *Int. J. Syst. Bacteriol.* 43:157–161.
22. **Collier, L., A. Balows, and M. Sussman (ed).** 1998. *Topley and Wilson's Microbiology and Microbial Infections*, 9th ed., vol. 2 and 3. Edward Arnold, London, England.
23. **Cosenza, B. J., and J. D. Podgwaite.** 1966. A new species of *Proteus* isolated from larvae of the gypsy moth *Porthetria dispar* (L.). *Antonie Leeuwenhoek J. Microbiol. Serol.* 32: 187–191.
24. **Crosa, J. H., D. J. Brenner, W. H. Ewing, and S. Falkow.** 1973. Molecular relationships among the salmonelleae. *J. Bacteriol.* 115:307–315.
25. **Dickey, R. S., and C. H. Zumoff.** 1988. Emended description of *Enterobacter cancerogenus* comb. nov. (formerly *Erwinia cancerogena*). *Int. J. Syst. Bacteriol.* 38:371–374.
26. **DuPont, H. L., S. B. Formal, R. B. Hornick, M. J. Snyder, J. P. Libonati, D. G. Sheahan, E. H. LaBrec, and J. P. Kalas.** 1971. Pathogenesis of *Escherichia coli* diarrhea. *N. Engl. J. Med.* 285:1–9.
27. **Ewing, W. H.** 1986. *Edwards and Ewing's Identification of Enterobacteriaceae*, 4th ed. Elsevier Science Publishing Co., New York, N.Y.
28. **Ewing, W. H., B. R. Davis, M. A. Fife, and E. F. Lessel.** 1973. Biochemical characterization of *Serratia liquefaciens* (Grimes and Hennerty) Bascomb et al. (formerly *Enterobacter liquefaciens*) and *Serratia rubidaea* comb. nov. and designation of type and neotype strains. *Int. J. Syst. Bacteriol.* 23:217–225.
29. **Ewing, W. H., and M. A. Fife.** 1972. *Enterobacter agglomerans* (Beijerinck) comb. nov. (the *herbicola-lathyri* bacteria). *Int. J. Syst. Bacteriol.* 22:4–11.
30. **Ewing, W. H., A. J. Ross, D. J. Brenner, and G. R. Fanning.** 1978. *Yersinia ruckerii* sp. nov., the redmouth (RM) bacterium. *Int. J. Syst. Bacteriol.* 28:37–44.
31. **Farmer, J. J., III, M. A. Asbury, F. W. Hickman, D. J. Brenner, and The *Enterobacteriaceae* Study Group.** 1980. *Enterobacter sakazakii*: a new species of "*Enterobacteriaceae*" isolated from clinical specimens. *Int. J. Syst. Bacteriol.* 30:569–584.
32. **Farmer, J. J., III, and B. R. Davis.** 1985. H7 antiserum-sorbitol fermentation medium: a single tube screening medium for detecting *Escherichia coli* O157:H7 associated with hemorrhagic colitis. *J. Clin. Microbiol.* 22:620–625. (*Note*: This paper has a misprint in the formula for MacConkey sorbitol agar. The paper says to use 22.2 grams of MacConkey agar base; the correct amount is 40 grams, which is given in the instructions on the bottle.)
33. **Farmer, J. J., III, B. R. Davis, F. W. Hickman-Brenner, A. McWhorter, G. P. Huntley-Carter, M. A. Asbury, C. Riddle, H. G. Wathen, C. Elias, G. R. Fanning, A. G. Steigerwalt, C. M. O'Hara, G. K. Morris, P. B. Smith, and D. J. Brenner.** 1984. Biochemical identification of new species and biogroups of *Enterobacteriaceae* isolated from clinical specimens. *J. Clin. Microbiol.* 21:46–76.
34. **Farmer, J. J., III, G. R. Fanning, B. R. Davis, C. M. O'Hara, C. Riddle, F. W. Hickman-Brenner, M. A. Asbury, V. Lowery, and D. J. Brenner.** 1984. *Escherichia fergusonii* and *Enterobacter taylorae*: two new species of *Enterobacteriaceae* isolated from clinical specimens. *J. Clin. Microbiol.* 21:77–81.
35. **Farmer, J. J., III, G. R. Fanning, G. P. Huntley-Carter, B. Holmes, F. W. Hickman, C. Richard, and D. J. Brenner.** 1981. *Kluyvera*: a new (redefined) genus in the family *Enterobacteriaceae*: identification of *Kluyvera ascorbata* sp. nov. and *Kluyvera cryocrescens* sp. nov. in clinical specimens. *J. Clin. Microbiol.* 13:919–933.
36. **Farmer, J. J., III, J. H. Jorgensen, P. A. D. Grimont, R. J. Akhurst, G. O. Poinar, Jr., E. Ageron, G. V. Pierce, J. A. Smith, G. P. Carter, K. L. Wilson, and F. W. Hickman-Brenner.** 1989. *Xenorhabdus luminescens* (DNA hybridization group 5) from human clinical specimens. *J. Clin. Microbiol.* 27:1594–1600.
37. **Farmer, J. J., III, A. C. McWhorter, D. J. Brenner, and G. K. Morris.** 1984. The *Salmonella-Arizona* group of *Enterobacteriaceae*: nomenclature, classification and reporting. *Clin. Microbiol. Newsl.* 6:63–66.
38. **Farmer, J. J., III, N. K. Sheth, J. A. Hudzinski, H. D. Rose, and M. F. Asbury.** 1982. Bacteremia due to *Cedecea neteri* sp. nov. *J. Clin. Microbiol.* 16:775–778.
39. **Farmer, J. J., III, J. G. Wells, P. M. Griffin, and I. K. Wachsmuth.** 1987. *Enterobacteriaceae* infections, p. 233–296. *In* B. B. Wentworth (ed.), *Diagnostic Procedures for Bacterial Infections*, 7th ed. American Public Health Association, Washington, D.C.
40. **Ferragut, C., D. Izard, F. Gavini, K. Kersters, J. DeLey, and H. Leclerc.** 1983. *Klebsiella trevisanii*: a new species from water and soil. *Int. J. Syst. Bacteriol.* 33:133–142.
41. **Ferragut, C., D. Izard, F. Gavini, B. Lefebre, and H. Leclerc.** 1981. *Buttiauxella*, a new genus of the family *Enterobacteriaceae*. *Zentralbl. Bakteriol. Parasitenkd. Infektionskr. Hyg. Abt. 1. Orig. Reihe C* 2:33–44.
42. **Gavini, F., C. Ferragut, D. Izard, P. A. Trinel, H. Leclerc, B. Lefebvre, and D. A. A. Mossel.** 1979. *Serratia fonticola*, a new species from water. *Int. J. Syst. Bacteriol.* 29:92–101.
43. **Gavini, F., J. Mergaert, A. Beji, C. Mielcarek, D. Izard, K. Kersters, and J. De Ley.** 1989. Transfer of *Enterobacter agglomerans* (Beijerinck 1888) Ewing and Fife 1972 to *Pantoea* gen. nov. as *Pantoea agglomerans* comb. nov. and description of *Pantoea dispersa* sp. nov. *Int. J. Syst. Bacteriol.* 39:337–345.
44. **Grimont, P. A. D., and E. Ageron.** 1989. *Enterobacter cancerogenus* (Urosevic, 1966) Dickey and Zumoff 1988, a senior subjective synonym of *Enterobacter taylorae* Farmer et al. (1985). *Res. Microbiol.* 140:459–465.
45. **Grimont, P. A. D., J. J. Farmer III, F. Grimont, M. A. Asbury, D. J. Brenner, and C. Deval.** 1983. *Ewingella americana* gen. nov., sp. nov., a new *Enterobacteriaceae* isolated from clinical specimens. *Ann. Microbiol. (Inst. Pasteur)* 134A:39–52.
46. **Grimont, P. A. D., F. Grimont, J. J. Farmer III, and M. A. Asbury.** 1981. *Cedecea davisae* gen. nov., sp. nov., and *Cedecea lapagei* sp. nov., new *Enterobacteriaceae* from clinical specimens. *Int. J. Syst. Bacteriol.* 31:317–326.
47. **Grimont, P. A. D., F. Grimont, C. Richard, B. R. Davis, A. G. Steigerwalt, and D. J. Brenner.** 1978. Deoxyribonucleic acid relatedness between *Serratia plymuthica* and other *Serratia* species, with a description of *Serratia odorifera* sp.

nov. (type strain: ICPB 3995). *Int. J. Syst. Bacteriol.* **28:** 453–463.

48. **Grimont, P. A. D., F. Grimont, C. Richard, and R. Sakazaki.** 1980. *Edwardsiella hoshinae*, a new species of *Enterobacteriaceae*. *Curr. Microbiol.* **4:**347–351.
49. **Grimont, P. A. D., F. Grimont, and M. P. Starr.** 1979. *Serratia ficaria* sp. nov., a bacterial species associated with smyrna figs and the fig wasp *Blastophaga psenes*. *Curr. Microbiol.* **2:**277–282.
50. **Grimont, P. A. D., T. A. Jackson, E. Ageron, and M. J. Noonan.** 1988. *Serratia entomophila* sp. nov. associated with amber disease in the New Zealand grass grub *Costelytra zealandica*. *Int. J. Syst. Bacteriol.* **38:**1–6.
51. **Harris, J. C., H. L. DuPont, and R. B. Hornick.** 1971. Fecal leukocytes in diarrheal illness. *Ann. Intern. Med.* **76:** 697–703.
52. **Hawke, J. P., A. C. McWhorter, A. G. Steigerwalt, and D. J. Brenner.** 1981. *Edwardsiella ictaluri* sp. nov., the causative agent of enteric septicemia of catfish. *Int. J. Syst. Bacteriol.* **31:**396–400.
53. **Hickman, F. W., and J. J. Farmer III.** 1978. *Salmonella typhi*: identification, antibiograms, serology, and bacteriophage typing. *Am. J. Med. Technol.* **44:**1149–1159.
54. **Hickman, F. W., J. J. Farmer III, A. G. Steigerwalt, and D. J. Brenner.** 1980. Unusual groups of *Morganella* ("*Proteus*") *morganii* isolated from clinical specimens: lysine-positive and ornithine-negative biogroups. *J. Clin. Microbiol.* **12:**88–94.
55. **Hickman, F. W., A. G. Steigerwalt, J. J. Farmer III, and D. J. Brenner.** 1982. Identification of *Proteus penneri* sp. nov., formerly known as *P. vulgaris* indole negative or as *Proteus vulgaris* biogroup 1. *J. Clin. Microbiol.* **15:** 1097–1102.
56. **Hickman-Brenner, F., J. J. Farmer III, A. G. Steigerwalt, and D. J. Brenner.** 1983. *Providencia rustigianii*: a new species in the family *Enterobacteriaceae* formerly known as *Providencia alcalifaciens* biogroup 3. *J. Clin. Microbiol.* **15:** 1057–1060.
57. **Hickman-Brenner, F. W., G. P. Huntley-Carter, G. R. Fanning, D. J. Brenner, and J. J. Farmer III.** 1985. *Koserella trabulsii*, a new genus and species of *Enterobacteriaceae* formerly known as Enteric Group 45. *J. Clin. Microbiol.* **21:**39–42.
58. **Hickman-Brenner F. W., G. P. Huntley-Carter, Y. Saitoh, A. G. Steigerwalt, J. J. Farmer III, and D. J. Brenner.** 1984. *Moellerella wisconsensis*, a new genus and species of *Enterobacteriaceae* found in human stool specimens. *J. Clin. Microbiol.* **19:**460–463.
59. **Hickman-Brenner, F. W., M. P. Vohra, G. P. Huntley-Carter, G. R. Fanning, V. A. Lowery III, D. J. Brenner, and J. J. Farmer III.** 1985. *Leminorella*, a new genus of *Enterobacteriaceae*: identification of *Leminorella grimontii* sp. nov. and *Leminorella richardii* sp. nov. found in clinical specimens. *J. Clin. Microbiol.* **21:**234–239.
60. **Hollis, D. G., F. W. Hickman, G. R. Fanning, J. J. Farmer III, R. E. Weaver, and D. J. Brenner.** 1981. *Tatumella ptyseos* gen. nov., sp. nov., a member of the family *Enterobacteriaceae* found in clinical specimens. *J. Clin. Microbiol.* **14:**79–88.
61. **Izard, D., C. Ferragut, F. Gavini, K. Kersters, J. DeLey, and H. Leclerc.** 1981. *Klebsiella terrigena*, a new species from soil and water. *Int. J. Syst. Bacteriol.* **31:**116–127.
62. **Izard, D., F. Gavini, and H. Leclerc.** 1980. Polynucleotide sequence relatedness and genome size among *Enterobacter intermedium sp. nov.* and the species *Enterobacter cloacae* and *Klebsiella pneumoniae*. *Zentralbl. Bakteriol. Parasitenkd. Infektionskr. Hyg. Abt. I Orig. Reihe C* **1:**51–60.
63. **Izard, D., F. Gavini, P. A. Trinel, and H. Leclerc.** 1979. *Rahnella aquatilis*, nouveau membre de la famille des *Enterobacteriaceae*. *Ann. Microbiol. (Inst. Pasteur)* **130A:** 163–177.
64. **Izard, D., F. Gavini, P. A. Trinel, and H. Leclerc.** 1981. Deoxyribonucleic acid relatedness between *Enterobacter cloacae* and *Enterobacter amnigenus* sp. nov. *Int. J. Syst. Bacteriol.* **31:**35–42.
65. **Jensen, K. T., W. Frederiksen, F. W. Hickman-Brenner, A. G. Steigerwalt, C. F. Riddle, and D. J. Brenner.** 1992. Recognition of *Morganella* subspecies, with proposal of *Morganella morganii* subspecies *morganii* subsp. nov. and *Morganella morganii* subspecies *sibonii* subsp. nov. *Int. J. Syst. Bacteriol.* **42:**613–620.
66. **Judicial Commission of the International Committee on Systematic Bacteriology.** 1993. Rejection of the name *Citrobacter diversus* Werkman and Gillen. *Int. J. Syst. Bacteriol.* **43:**392.
67. **Kandolo, K., and G. Wauters.** 1985. Pyrazinamidase activity in *Yersinia enterocolitica* and related organisms. *J. Clin. Microbiol.* **21:**980–982.
68. **Karmali, M. A.** 1989. Infection by verocytotoxin-producing *Escherichia coli*. *Clin. Microbiol. Rev.* **2:**15–38.
69. **Kauffmann, F.** 1966. *The Bacteriology of Enterobacteriaceae*. The Williams & Wilkins Co., Baltimore, Md.
70. **Kosako, Y., K. Tamura, R. Sakazaki, and K. Miki.** 1996. *Enterobacter kobei* sp. nov., a new species of *Enterobacteriaceae* resembling *Enterobacter cloacae*. *Curr. Microbiol.* **33:** 261–265.
71. **Krieg, N. R., and J. G. Holt (ed.).** 1984. *Bergey's Manual of Systematic Bacteriology*, vol. 1, p. 408–516. The Williams & Wilkins Co., Baltimore, Md.
72. **Lannigan, R., and Z. Hussian.** 1993. Wound isolate of *Salmonella typhimurium* that became chlorate resistant after exposure to Dakin's solution: concomitant loss of hydrogen sulfide production, gas production, and nitrate reduction. *J. Clin. Microbiol.* **31:**2497–2498.
73. **Le Minor, L., M. Y. Popoff, B. Laurent, and D. Hermant.** 1986. Individualisation d'une septième sous-espèce de *Salmonella*: *S. choleraesuis* subsp. *indica* subsp. nov. *Ann. Microbiol. (Inst. Pasteur)* **137B:**211–217.
74. **Li, K., J. J. Farmer III, and A. Coppola.** 1974. A novel type of resistant bacteria induced by gentamicin. *Trans. N. Y. Acad. Sci.* **36:**369–396.
75. **McWhorter, A. C., R. L. Haddock, F. A. Nocon, A. G. Steigerwalt, D. J. Brenner, S. Aleksic, J. Bockemuhl, and J. J. Farmer III.** 1991. *Trabulsiella guamensis*, a new genus and species of the family *Enterobacteriaceae* that resembles *Salmonella* subgroups 4 and 5. *J. Clin. Microbiol.* **29:** 1480–1485.
76. **McWhorter-Murlin, A. C., and F. W. Hickman-Brenner.** 1994. *Identification and Serotyping of* Salmonella *and an Update of the Kauffmann-White Scheme*; *Appendix A, Kauffmann-White Scheme, Alphabetical List of* Salmonella *Serotypes* (updated 1994); *Appendix B, Kauffmann-White Scheme, List of* Salmonella *Serotypes by O Group* (updated 1994). Foodborne and Diarrheal Diseases Laboratory Section, Centers for Disease Control and Prevention, Atlanta, Ga.
77. **Müller, H. E., D. J. Brenner, G. R. Fanning, P. A. D. Grimont, and P. Kämpfer.** 1996. Emended description of *Buttiauxella agrestis* with recognition of six new species of *Buttiauxella* and two new species of *Kluyvera*: *Buttiauxella ferragutiae* sp. nov., *Buttiauxella gaginiae* sp. nov., *Buttiauxella brennerae* sp. nov., *Buttiauxella izardii* sp. nov., *Buttiauxella noackiae* sp. nov., *Buttiauxella warmboldiae* sp. nov., *Kluyvera cochleae* sp. nov., and *Kluyvera georgiana* sp. nov. *Int. J. Syst. Bacteriol.* **46:**50–63.
78. **Müller, H. E., C. M. O'Hara, G. R. Fanning, F. W. Hickman-Brenner, J. M. Swenson, and D. J. Brenner.** 1986.

*Providencia heimbachae*, a new species of *Enterobacteriaceae* isolated from animals. *Int. J. Syst. Bacteriol.* **36**:252–256.

79. **O'Hara, C. M., A. G. Steigerwalt, B. C. Hill, J. J. Farmer III, G. R. Fanning, and D. J. Brenner.** 1989. *Enterobacter hormaechei*, a new species of the family *Enterobacteriaceae* formerly known as enteric group 75. *J. Clin. Microbiol.* **27**: 2046–2049.
80. **Pickering, L. K., H. L. DuPont, J. Olarte, R. Conklin, and C. Ericsson.** 1977. Fecal leucocytes in enteric infections. *Am. J. Clin. Pathol.* **68**:562–565.
81. **Popoff, M. Y., and L. Le Minor.** 1997. *Antigenic Formulas of the* Salmonella *Serovars*, 7th revision. WHO Collaborating Centre for Reference and Research on *Salmonella*. Institut Pasteur, Paris, France.
82. **Popoff, M. Y., and L. Le Minor.** 1997. *Guidelines for the Preparation of* Salmonella *Antisera*. WHO Collaborating Centre for Reference and Research on *Salmonella*. Institut Pasteur, Paris, France.
83. **Reeves, M. W., G. M. Evins, A. A. Heiba, B. D. Plikaytis, and J. J. Farmer III.** 1989. Clonal nature of *Salmonella typhi* and its genetic relatedness to other salmonellae as shown by multilocus enzyme electrophoresis, and proposal of *Salmonella bongori* comb. nov. *J. Clin. Microbiol.* **27**: 313–320.
84. **Riley, G., and S. Toma.** 1989. Detection of pathogenic *Yersinia enterocolitica* by using Congo red-magnesium oxalate agar medium. *J. Clin. Microbiol.* **27**:213–214.
85. **Sakazaki, R., K. Tamura, Y. Kosako, and E. Yoshizaki.** 1989. *Klebsiella ornithinolytica* sp. nov., formerly known as ornithine-positive *Klebsiella oxytoca*. *Curr. Microbiol.* **18**: 201–206.
86. **Schaberg, D. R.** 1991. Major trends in the microbial etiology of nosocomial infections. *Ann. Intern. Med.* **91**(Suppl. 3B):72S–75S.
87. **Sprierings, G., C. Ockhuijsen, H. Hofstra, and J. Tommassen.** 1993. Polymerase chain reaction for the specific detection of *Escherichia coli/Shigella*. *Res. Microbiol.* **144**: 557–564.
88. **Tamura, K., R. Sakazaki, Y. Kosako, and E. Yoshizaki.** 1986. *Leclercia adecarboxylata* gen. nov., comb. nov., formerly known as *Escherichia adecarboxylata*. *Curr. Microbiol.* **13**:179–184.
89. **Thomas, G. M., and G. O. Poinar, Jr.** 1979. *Xenorhabdus* gen. nov., a genus of entomopathogenic nematophilic bacteria of the family *Enterobacteriaceae*. *Int. J. Syst. Bacteriol.* **29**:352–360.
90. **Ursing, J., D. J. Brenner, H. Bercovier, G. R. Fanning, A. G. Steigerwalt, J. Brault, and H. H. Mollaret.** 1980. *Yersinia frederiksenii*: a new species of *Enterobacteriaceae* composed of rhamnose-positive strains (formerly called atypical *Yersinia enterocolitica* or *Yersinia enterocolitica*-like). *Curr. Microbiol.* **4**:213–217.
91. **Wadstrom, T., A. Aust-Kettis, D. Habte, J. Holmgren, G. Meeuwisse, R. Mollby, and O. Soderlind.** 1976. Enterotoxin-producing bacteria and parasites in stool of Ethiopian children with diarrhoeal disease. *Arch. Dis. Child.* **51**:865–870.
92. **Wauters, G., M. Janssens, A. G. Steigerwalt, and D. J. Brenner.** 1988. *Yersinia mollaretii* sp. nov. and *Yersinia bercovieri* sp. nov., formerly called *Yersinia enterocolitica* biogroups 3A and 3B. *Int. J. Syst. Bacteriol.* **38**:424–429.
93. **Wells, J. G., B. R. Davis, I. K. Wachsmuth, L. W. Riley, R. S. Remis, R. Sokolow, and G. K. Morris.** 1983. Laboratory investigation of hemorrhagic colitis outbreaks associated with a rare *Escherichia coli* serotype. *J. Clin. Microbiol.* **18**:512–520.